

OBSERVATIONS ON HAEMOLYTIC DISEASE

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A Thesis for the Degree of Doctor of
Medicine in the University of Edinburgh

March 1963



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FOREWORD

The concept of anaemia due to excessive red cell destruction was first advanced at the end of last century. Since then the development of methods for measuring the red cell survival time, sophistication of serological techniques for detection of erythrocyte antibodies and discoveries regarding the nature of inborn red cell defects have clearly shown that many causes for haemolysis exist. Increased red cell destruction is now known to be an accompaniment of many diseases, often in the absence of the classical signs of anaemia, reticulocytosis and acholuric jaundice. Moreover, it has been found that in only a few conditions are therapeutic measures available which will ameliorate or arrest the haemolytic process.

The thesis describes studies on the pathogenesis, natural history and management of haemolytic disease and has been divided into five chapters. The first chapter outlines briefly the evolution of knowledge leading to a definition of haemolysis and to a classification of the important haemolytic disorders. In the second chapter the methods of investigation have been discussed and particular emphasis has been given to the technique of red cell survival measurement, to the possibility of evaluating the role of the spleen in the haemolytic process and to the interpretation/

interpretation of results. It was decided to restrict the main considerations of the thesis to observations in the three groups of haemolytic disease that are seen most frequently in Great Britain, namely autoimmune haemolytic anaemia, symptomatic non-immune haemolytic anaemia (as encountered in leukaemia, reticulosis and myeloid metaplasia) and congenital spherocytosis; accordingly the third, fourth and fifth chapters deal respectively with these subjects and data from seventy-six patients have been used.

In each series, the modes of presentation of disease, the clinical features and the diagnostic indicators have been reviewed. The principal contributions are concerned with the assessment of different forms of treatment in autoimmune haemolytic anaemia, leading to the use of anti-neoplastic drugs in fulminating disease, the effect of splenic irradiation in the symptomatic non-immune group and observations on the mode of inheritance of disease and on the action of the spleen in congenital spherocytosis. Other studies have dealt with the utilisation of haemopoietic factors in haemolytic anaemia, the uptake of chromium-51 labelled red cells by different body tissues obtained at autopsy and the incidental finding of hypocholesterolaemia in untreated congenital spherocytosis. The present methods for quantitating the splenic sequestration of radioactive labelled red cells are considered to be unsatisfactory and the use of a new index, termed the "Spleen/

"Spleen Number", has been proposed.

My interest in haemolytic disease began in 1955, when in collaborative studies with Dr. J. J. R. Duthie at the Rheumatic Diseases Unit, Northern General Hospital, Edinburgh, it was found that shortened red cell survival was one factor in the anaemia complicating rheumatoid arthritis. On return to the Department of Medicine, University of Edinburgh, in 1956, the interest widened under the guidance of Sir Stanley Davidson, particularly after learning the procedure for labelling red corpuscles with radioactive chromium. Then in 1958-59, I spent twelve months at the Memorial Center for Cancer and Allied Diseases, New York; this afforded a unique opportunity for studying the haemolytic process that accompanies leukaemia and lymphoma, and during this period I received much helpful criticism and advice from Dr. Lloyd F. Craver, Head of the Lymphoma Service and Dr. Allyn B. Ley, Head of the Hematology Service. Subsequently I have been indebted to Professor K. W. Donald for encouraging me to continue clinical and experimental studies in this field.

I wish to thank the many Physicians in Edinburgh and New York who, during the past eight years, have allowed me to investigate patients under their care. I also acknowledge the important contribution to diagnosis and to the assessment of each patient's progress that has been made by the routine haematological and biochemical departments in the/

the two centres. Two colleagues require particular mention. First, Professor R. H. Girdwood from whom I have learned a great deal about haematology, many of whose cases are featured in the thesis and who has kindly undertaken serum assays for folic acid and vitamin B₁₂. Second, Dr. R. A. Cumming who has supplied all the information regarding serological studies in the Edinburgh cases and whose efficient Blood Transfusion Service has contributed in large measure to the survival of a number of the patients.

THE LANDMARKS OF PROGRESS1. 1. Historical

The development of the microscope in the seventeenth century led to the first accounts of red corpuscles in blood. Swammerdam (1658) may have discovered them but the best of the early descriptions is attributed to a little Dutch grocer (Leuwonhoeck 1674) who made the practice of examining his own blood every day and regulating his habits from what he found. It was probably not until the time of Hewson, 100 years later, that the erythrocytes were known to be biconcave discs, not "ruddy globules" as Leuwonhoeck had believed; in dissertations to the Royal Society Hewson reported their behaviour in water and in hypertonic saline.

Boyle (1661), through his own observations, rejected the earlier view that the function of breathing was to cool the blood. He showed that if a burning candle and a mouse or sparrow were placed in a chamber from which air were gradually exhausted, then the animal would die almost at the same time as the candle went out. Lavoisier prior to his execution seems to have been near to interpreting the respiratory process, but it is generally believed that the role of the red corpuscle in oxygen transport was first propounded correctly in an important paper to the British Association by von Liebig in 1852. Fifteen years later, Hoppe-Selyer confirmed that haemoglobin had the capacity for reversible combination with oxygen and carbon dioxide as had been suggested/

suggested in von Liebig's theory.

During this time search was being made for the source of erythrocytes. Hewson believed they originated from leucocytes, Hayem from platelets, Addison the adrenals, Reichert the liver and Kölliker the spleen. It was Neumann who in 1868 demonstrated that mammalian red corpuscles arise from colourless nucleated elements in the bone marrow. Although laborious techniques were introduced for counting blood cells and for measuring the colouring matter of blood in the middle of the nineteenth century (Vierordt 1852, Welcker 1854), marrow biopsy was not performed during life until the beginning of the present century (Panese 1903) and marrow aspiration, as is now practised, not until after the work of Arinkin in 1929.

Lieutaud, a physician of Versailles, popularised the term anaemia, "an exhaustion of the blood vessels", in a textbook published in 1759. In the previous century, long before iron was known to be present in blood Sydenham had however been using chalybeates in the treatment of chlorosis; the modern views on the correction of iron deficiency date from the introduction of the "veritable pills" of Dr. Blaud in 1832. The clinical features of pernicious anaemia were first fully described by Addison in 1855 although others (Combe 1823, Andral 1823) probably reported this condition much earlier. The concept of "aplastic anaemia" was introduced by Ehrlich in 1888.

Two Belgian physicians (Vanlair and Masius 1871) in their description of a patient with "microcythaemia" were evidently the first to propose that anamia might be due to the abnormally rapid destruction of red blood cells (Crosby 1952). They suggested that their patient's jaundice was due to the excess of bile pigment formed when haemoglobin was released from the "senile" red cells.

According to Crosby (1952) it was Hunter (1901) who introduced the term "haemolytic". From the studies of others and from his own experiments on chemically induced anaemia in dogs he concluded that small spherical microcytes, as had been seen and illustrated by Vanlair and Masius, "mark the anaemia as due to excessive destruction of blood and not to deficient formation (and) that they denote the anaemia to be haemolytic, not haemogenic in its origin" (Dacie 1960).

1. 2. The recognition of acquired haemolytic anaemias

A familial form of jaundice was reported by several authors towards the end of last century and the account of Wilson and Stanley (1893) undoubtedly referred to, and described in some detail, congenital spherocytosis. Minkowski (1900) then clearly separated haemolytic icterus from that due to biliary obstruction. Hayem (1898) and later Widal, Abrami and Brule (1907) drew attention to another disorder that seemed to be distinct from congenital spherocytosis, /

spherocytosis, in which autohaemagglutination was frequently noted and in which anaemia could be severe and illness profound. Furthermore Chauffard and Troisier (1908) described cases in which haemolysins were present in the patients' serum. Numerous studies were reported by Chauffard and by Widal and his colleagues but were overlooked and when Lederer in 1925 described three cases of acute and severe haemolytic anaemia associated with the presence of serum haemolysins this was hailed as a new discovery. However it was not until the report of Dameshek and Schwartz (1940) that the existence of an acquired type of haemolytic anaemia as distinct from congenital disease was finally accepted. These authors undertook extensive studies in four patients in whom acute anaemia appeared to be due to abnormal haemolysins, a phenomenon they were able to repeat in experimental animals, and just as Lederer's patients had responded dramatically to blood transfusion, theirs responded dramatically to splenectomy. Dameshek and Schwartz are also responsible for emphasising that spherocytosis and increased fragility of spherocytes in hypotonic solutions of saline may occur in both the congenital and acquired varieties of haemolytic anaemia and that these findings are not, as Naegeli (1931) taught, pathognomonic of the former.

The next advance followed two apparently unrelated findings. One of these concerned haemolytic disease of the/

the newborn, for which many theories had been proposed; it fell to Levine, Katzin and Burnham (1941) to make the significant observation that there was a haemagglutinin in the serum of mothers with affected children which agglutinated the red cells of the fathers as well as those of the children. The other, which had been slightly earlier, was the discovery by Landsteiner and Wiener (1937, 1941) of the Rh system of blood group agglutinogens. Soon afterwards it was shown that anti-Rh was a potent cause of haemolytic transfusion reactions (Wiener and Peters 1940, 1941) and that Rh incompatibility between mother and child was the most frequent cause of erythroblastosis foetalis.

Experience with Rh sensitisation revealed that Rh antibodies might not behave in the same way as the naturally occurring red cell antibodies (anti-A, anti-B). They were termed "incomplete" antibodies because they appeared to combine specifically with Rh-positive erythrocytes, "coating" the surface of the cell without producing in vitro agglutination and "blocking" agglutination of these cells, when exposed to appropriate anti-sera, in saline suspensions. It is now known that this form of sensitisation can occur with antibodies against a variety of blood group substances other than the Rh system and in addition to its implication in erythroblastosis foetalis and blood transfusion it appears to be the cause of a large proportion of cases of acquired haemolytic anaemia.

One of the greatest difficulties, initially, was the recognition and characterisation of these "coating antibodies" until Coombs, Mourant and Race (1945) using the fact that the antibodies were globulins were able to induce their in vitro detection by the exposure of sensitised cells to anti-human globulin serum (prepared by immunising a rabbit). Although other tests have been introduced Coombs' test remains a major contribution to the study of haemolytic disorders and is still the most generally useful procedure when a haemolytic process is believed to be due to red cell antibodies.

Despite intensive study the precise mechanism of action of red cell antibodies in shortening red cell survival is not certain and as Wintrobe (1961) suggests a number of activities are probably involved. One reason for this that was known fifty years ago (Muir and McNee 1911) and which has more recently been confirmed (Wasastjerna 1948) is the discrepancy between the in vitro and vivo behaviour of anti-erythrocytic serum. Not only are there great differences in the rate of reaction in the two situations but antibodies that appear to cause haemolysis in the intact animal may only cause agglutination in the test tube. Erythrophagocytosis of sensitised cells certainly occurs (Zinkham and Diamond 1952) but seems unlikely to be quantitatively important. Although agglutination of coated cells is difficult/

difficult to secure in vitro, agglutination is considered to be a fundamental factor and has been seen to occur intravascularly in animals and in man (Wasastjerna, Pisciotto and Dameshek 1954a, 1954b). Agglutination is the fate of sensitised cells injected experimentally and this is probably a first step leading to their sequestration in the spleen and elsewhere (Jandl, Jones and Castle, 1957).

There is much conflicting data regarding the spleen which will be referred to again (p. 80). An important feature of experimental immune haemolytic anaemias is that blood destruction is much less in animals after splenectomy than in normal animals (Wasastjerna 1948). In man studies using radioactive labelled red corpuscles also indicate that the spleen may play a major role in certain antibody-induced haemolytic anaemias (Jandl, Greenberg, Yonemoto and Castle 1956, Schoesser, Korst, Clatanoff and Schilling 1957); other work suggests, however, that coarsely agglutinated cells may be removed from the circulation chiefly by the liver and lung (Jandl 1955).

It may yet be shown that the most important sequel of red cell sensitisation is critical interference with red cell metabolism which renders the corpuscle more susceptible to destruction by other mechanisms. Allusions to the results of early experiments which support this hypothesis have been made by Pranker (1959). One visible result that has been mentioned is spherocytosis and convincing evidence of red cell damage is obtained in the work of Sharpsteen/

Sharpsteen (1955) who showed that coating by antibody significantly reduced the tensile strength of the red cell membrane.

1. 3. Enlargement of knowledge of intrinsic red cell defects

That spherocytosis might not be the only inherited red cell defect which could give rise to anaemia was suggested by the report of Herrick (1910) who found sickle-shaped red corpuscles in a patient with severe anaemia; the development of sickling in vitro was demonstrated by Emmel (1917); the inheritance of the corpuscular defect was established by Huck in 1923 and in Sydenstricker's paper in 1924 it was realised that "sickle cell anaemia" was a familial disease which affected the negro race. Hahn (1928) and later Sherman (1940) showed clearly that the phenomenon was enhanced in atmospheres of low oxygen tension and was favoured by lowering pH and by increasing the temperature to that of the body, while exposure to oxygen or carbon monoxide restored the circular form of sickled erythrocytes.

During the period of these discoveries Cooley and Lee (1925) described an anaemia characterised by "target" cells in the blood, splenomegaly and skeletal changes. They considered the anaemia to be of haemolytic type (Cooley, Witwer and Lee 1927) but were also concerned with the obvious defects in haemopoiesis (Cooley and Lee 1932) which is now regarded as the more important factor. The familial/

familial nature of the disease was established, serious and benign forms were observed and the incidence appeared to be in people of Mediterranean origin and in the Far East.

The first indication of specific biochemical red cell abnormalities in some of the haemolytic disorders came in 1949 when Pauling, Itano, Singer and Wells showed that haemoglobin from sickle cells had a different electrophoretic mobility from that of normal adult haemoglobin. Numerous reports of other variants of haemoglobin followed (known by different letters of the alphabet and by various eponyms) and since the haem moiety was the same in each, alteration in the globin part of haemoglobin came to be regarded as the abnormality. The validity of this concept of "molecular disease" was proved by Ingram in 1956 who, in a series of ingenious experiments using chromatography of tryptic digests of haemoglobin, showed that the difference between normal haemoglobin and sickle cell haemoglobin lay in the amino acid sequence in one of the polypeptide chains of globin. Further work has established that similar defects occur in several haemoglobinopathies while in others there is absence of one or both normal adult polypeptide chains; in haemoglobin-H for example only β chains are present but these appear to be normal (Jones, Schroeder, Balog and Vinograd 1959).

A notable advance followed investigation into the pathogenesis/

pathogenesis of the haemolytic anaemia which accompanies the giving of the anti-malarial drugs, pamaquine and primaquine, to susceptible subjects (Earle, Bigelow, Zubrod and Kane 1948, Dern, Weinstein, Le Roy, Talmage and Alving 1954). Negroes were found to be the most frequently affected (although it is now known that susceptibility is also found in Sephardic Jews, certain Arab groups and Caucasians of Italian and Greek extraction), the abnormality clearly resided in the erythrocytes of sensitive subjects and haemolysis was self limiting because only the older cells in the patients' circulation appeared to be destroyed. These findings led to the belief that sensitivity to these drugs might be related to deficiency in an enzyme system which decreases in activity as the red cell ages. Beutler, Dern, Larkin, Flanagan and Alving (1955) demonstrated that primaquine sensitive erythrocytes were uniformly deficient in reduced glutathione. Shortly afterwards Carson, Flanagan, Ickes and Alving (1956) presented evidence that this abnormality was associated with a deficiency of glucose-6-phosphate dehydrogenase activity; one action of this enzyme is known to be the maintenance of substrates necessary to reduce oxidised glutathione. Lately it has been demonstrated that this red cell enzyme defect is present in patients who show haemolysis when exposed to a wide range of drugs and chemicals, particularly aniline derivatives, as well as in those who are sensitive to the bean or the pollen/

pollen of the fava plant. It has still to be determined whether depletion of reduced glutathione plays a primary role in the destruction of sensitive cells or whether this is only a convenient indicator of more important deficiencies (Wintrobe 1961).

Reports of various other metabolic abnormalities in the cells of patients with congenital haemolytic anaemia are now numerous. A most exhaustive study by Erickson and her colleagues (Erickson, Williams, Hummel, Lee and Macey 1937; Erickson, Williams, Hummel and Macey) and later by Harris, Prankerd and Westerman (1958), demonstrated an increase in all groups of lipids in the red corpuscles in sickle cell anaemia; thalassaemic cells on the other hand show a marked deficit of lipid, especially of cerebroside, with some increase in phospholipid. Others have demonstrated that in the erythrocytes in congenital spherocytosis there is a significantly higher proportion of lysophosphatidyl ethanolamine and lower proportion of phosphatidyl ethanolamine than in normal cells (Allison, Kates and James 1960). In paroxysmal nocturnal haemoglobinuria (PNH) the haemolytic process is undoubtedly complex. Experimental evidence suggests that the fault in this condition is also in the red corpuscles (Dacie 1949). Differing abnormalities in the lipoprotein of the cells have been reported by Harris, Prankerd and Westerman (1957) and by Munn and Crosby (1957). The former group found a marked specific deficit of phosphatidyl/

phosphatidyl-choline. Reduction in acetylcholinesterase activity has also been observed (Hartmann and Auditore 1959); this appears to be a consistent finding in severe and moderately severe cases (Metz, Bradlow, Lewis and Dacie 1960).

The nature of the corpuscular defect in congenital spherocytosis is a study of particular interest. Using radioactive phosphorus it is found that there is a smaller flux of ^{32}P into adenosine triphosphate and 2,3-diphosphoglyceric acid than in normal cells (Prankerd, Altman and Young 1955, 1960) indicating a disturbance in phosphorylation. A number of enzyme deficiencies have been suggested but are still to be proved.

The impression is gained that while the pathogenesis of none of the congenital haemolytic disorders has yet been explained, the evidence accumulating strongly suggests that these conditions will ultimately be shown to be true inborn errors of metabolism due to defects of enzymes or other vital substances. Moreover, the present enquiries into red cell metabolism suggest that red cell ageing may well be the sequel of utilisation of non-replicating enzymes, particularly those concerned in glycolysis (Hirschberg and Banks 1958, Bernstein 1959).

1. 4. The contribution of red cell survival measurement

It is uncertain when it was first appreciated that red corpuscles/

corpuscles had a finite survival time. The first studies into the life span of erythrocytes that are known were made 100 years ago when various investigators (Marfels and Moleschott 1856, Brown-Sequard 1867) examined the question by injecting nucleated red corpuscles from birds and frogs into animals having non-nucleated corpuscles and measuring the rate of disappearance of transfused nucleated corpuscles from the recipient's circulation. We now know that because of species' difference these experiments cannot have yielded valuable information.

The first technically good estimates of red cell survival in man are believed to have been made by Ashby (1919) who developed a method used a few years earlier in bulls (Todd and White 1911). The method depended on the transfusion to the subject under study, of blood that was compatible but immunologically identifiable (e.g. Group 'O' blood to a Group 'A' recipient). Subsequently the rate of disappearance of donor red cells could be followed by agglutinating the recipient's red cells in serial samples of peripheral blood using appropriate antisera and enumerating the free donor cells that remained. While the technique is tedious and subject to certain criticisms, it has contributed much to the understanding of haemolytic disorders; improvements made it possible to utilise the MN and Rh blood group systems and it was widely employed until about ten years ago, since which time it has been largely/

largely superseded by methods using radioactive isotopes (p.41).

Although investigators (Callender, Powell and Witts 1947, Berlin 1951, Hurley and Weisman 1953, Eadie, Brown and Curtis 1955) have shown a slight deviation from a straight line in a small number of patients, when the graph of percentage survival is plotted against time, it has been found that in health the disappearance of normal red cells from the circulation of a normal individual is usually a linear function and that the red corpuscles have a life span in the region of 110 to 120 days. Approximately 0.8 per cent of the total number of corpuscles are destroyed each day and this form of elimination has been taken to indicate that the age of the red cells is the main factor in determining survival.

This orderly pattern of destruction does not however obtain in haemolytic disorders. In various types of acquired haemolytic anaemia, Loutit and Mollison (1946) and Mollison (1947) showed that the rate of elimination of transfused normal corpuscles was greatly accelerated. When the results of their observations were plotted the curve was exponential and not straight; the loss of transfused cells was at first very rapid and then slow. The implication was that red cell destruction was being determined by at least two factors, age and extrinsic injury/

injury to the normal donor cells. The rapid initial disappearance of transfused erythrocytes was due to the "random" elimination of young cells as well as old, and as the transfused cells became fewer their chance of survival improved.

In paroxysmal nocturnal haemoglobinuria, in which condition the main abnormality is believed to reside in the red cells, transfusion of the patient's cells to a normal subject also produces an exponential type of elimination of cells (Dacie and Mollison 1949). The red cells differ widely in their ability to survive suggesting that in this type of disease the important factor affecting life span, other than age, is individual variation of the red cells in their susceptibility to destruction. Dacie and Mollison (1943) had earlier obtained similar results for congenital spherocytosis cells; furthermore they had found that when cells from healthy subjects were transfused to patients with congenital spherocytosis their survival time was normal.

The accuracy of these early transfusion experiments has been confirmed, studies have been expanded and two important concepts have resulted. Firstly, it has been realised that there are two main groups of haemolytic disorder; there are on the one hand conditions in which the primary defect is in the red corpuscle; on the other hand are conditions in which the patient's erythrocytes are normal, there is no congenital abnormality and the haemolytic process is due to factors/

factors outwith the red corpuscle. Secondly, the use of measurements of red cell survival has led to our present definition of a haemolytic disorder as one in which the life span of the red cells is shortened; whatever else is clinically evident, this is the essential feature. The patient may not show splenomegaly or icterus or reticulocytosis and because of the marrow's ability to increase its productive capacity six to eight fold to compensate for increased blood destruction (Crosby and Akeroyd 1952, Crosby 1955), the patient may not be anaemic. It was this last observation that led Crosby (1955) to suggest that "haemolytic disease" was a more appropriate term than "haemolytic anaemia" or "haemolytic jaundice" for the group of disorders in which the main abnormality in the blood was excessively rapid destruction of red corpuscles. Studies of red cell survival have also shown that shortening of red cell life is found in a very wide range of conditions that had not formerly been regarded as causing haemolysis; these include infection, chronic inflammation (notably rheumatoid arthritis), hepatic cirrhosis, uraemia and malignant disease (Berlin, Waldmann and Weissman 1959, Dameshek and Schwartz 1959). In these disorders the normal indicators of overhaemolysis, namely acholuric jaundice and signs of increased activity of the bone marrow, are usually absent and it is probable that in these conditions reduced/

reduced red cell survival is only one factor and not the main factor in the genesis of anaemia.

1. 5. Classification of the haemolytic disorders

The landmarks of progress into the understanding of haemolytic disorders in the last twenty years have been discussed; the chief among these have been the development of serological techniques for the study of red cell antibodies, the extensive use of red cell survival measurements and the accelerating knowledge regarding metabolic defects in red corpuscles. A number of classifications for haemolytic disease have been suggested, based either on the severity of illness, e.g. into acute and chronic, or on inherited aspects of the illness (Dameshek and Schwartz 1940) into congenital and acquired. The best classification, utilising the present views on pathogenesis, recognises two main groups; an outline of this aetiological division is given in Table I.

In the first group (Group A) the red cells are held to be inherently normal, biochemically and morphologically. The cause for red cell injury and premature destruction is some extracorporeal factor. This may be infection, either bacterial (e.g. staphylococcal septicaemia) or parasitic (e.g. malaria). A number of chemicals (e.g. lead) or therapeutic agents (e.g. sulphones) may be implicated and severe thermal burns undoubtedly cause significant red cell damage (Moore, Peacock, Blakely and Cope 1946, James, Purnell/

TABLE 1

CLASSIFICATION OF HAEMOLYTIC DISORDERS

A. Due to mechanisms extrinsic to the red cell.

Infections

Chemical and physical agents

Red cell antibodies -

Symptomatic

Idiopathic

Cause undetermined -

With underlying disease

Without underlying disease

B. Due to defects within the red cell.

1. No other factor known to be implicated.

Congenital spherocytosis

Congenital elliptocytosis

Congenital non-spherocytic haemolytic anaemia

Thalassaemia

Haemoglobinopathies other than haemoglobin-S

2. An extracorporeal factor required for full expression.

Sickle cell disease

Paroxysmal nocturnal haemoglobinuria

Glucose-6-phosphate dehydrogenase deficiency

Purnell and Evans 1954). In a large and important group haemolysis appears to be due to antibodies produced by the patient against his own red cells. This may be associated with reticuloendothelial disease, particularly when lymphoid proliferation is predominant, (e.g. lymphosarcoma), or with connective tissue disease (e.g. lupus erythematosus). In approximately 50 per cent of patients no cause can be found for the spontaneous generation by a patient of antibodies against his own red cells, and the condition is described as "idiopathic autoimmune acquired haemolytic anaemia". There remain in Group A acquired haemolytic anaemias that have still to be explained; these are the ones that complicate chronic diseases (p. 133) and those that are found in patients with splenomegaly of miscellaneous causes and others without any guiding clue as to their aetiology.

Group B includes all the conditions in which the main abnormality is an intrinsic red cell defect. In some of these no other important factor has been found, e.g. congenital spherocytosis, while in others an extrinsic challenge appears to be necessary for the red corpuscle to become fully susceptible to destruction; in sickle cell disease this environmental factor is lowered oxygen tension, in PNH it is the metabolic changes associated with sleep and in glucose-6-phosphate dehydrogenase deficiency it is certain drugs, particularly aniline derivatives, and derivatives of the fava plant.

In/

In the chapters that follow the concept of two fundamental groups of haemolytic disease has been accepted and examples of most haemolytic diseases will be used to illustrate methods of investigation, such as tests of fragility and red cell survival measurement. The main considerations will, however, be restricted to three conditions in which the greatest experience has been gained.

CHAPTER TWO

CLINICAL MATERIAL AND METHODS OF INVESTIGATION2. 1. The patients.

Two varieties of haemolytic disorder are most commonly encountered in Northern Europe; these are congenital spherocytosis and autoimmune acquired haemolytic anaemia. For reasons of their racial incidence the haemoglobino-pathies and thalassaemia are seldom seen and the other specific entities are rare. Experience has shown, however, that reduction of red cell survival accompanies many systemic diseases; this is not due to identifiable red cell antibodies and the pathogenesis is ill understood. Reference has already been made to this occurrence in a number of chronic infections and inflammations (p. 18). Studies in rheumatoid arthritis (Alexander, Richmond, Roy and Duthie 1956, Richmond, Alexander, Potter and Duthie 1961) are typical of others and while a haemolytic process is present in this condition and will contribute to anaemia, it is unlikely to be an important clinical factor. There is, however, a different picture in patients with leukaemia, reticulosis and primary myeloid metaplasia; in this heterogeneous group there may be more striking reduction of red cell survival, particularly in those with splenomegaly, while the classical diagnostic indicators of haemolysis are absent. Excessive red cell destruction is frequently a major/

major cause for morbidity in these patients; the incidence is unknown because it seems to depend on the frequency with which the possibility of haemolysis is considered. For the purpose of this thesis, this last group will be referred to as suffering from "symptomatic non-immune haemolytic anaemia".

A special study has been made of the following:-

- (i) Autoimmune acquired haemolytic anaemia;
twenty-three patients,
- (ii) Symptomatic non-immune haemolytic anaemia;
twenty-seven patients,
- (iii) Congenital spherocytosis; twenty-six
patients.

All the cases in Groups (i) and (iii) and nine of the patients in Group (ii) have been investigated as in-patients or while attending hospitals in Edinburgh during the past eight years. Eighteen patients in Group (ii) were studied in the Memorial Hospital, New York, U.S.A., in the period 1958-59. The details regarding age, sex, clinical features and mode of presentation for each disease group will be given in the appropriate chapters.

While consideration of the natural history of the disease, pathogenesis of haemolysis and management of these seventy-six patients is the main substance of the thesis, reference will also be made to normal subjects, patients with miscellaneous conditions and patients with the rarer haemolytic/

haemolytic disorders in evaluation of osmotic fragility tests and measurements of red cell survival.

2. 2. Methods of investigation. Introduction.

Clinical suspicion of haemolytic disease usually arises from the presence of anaemia, acholuric jaundice and splenomegaly. Recurrent cholelithiasis, particularly when only pigment stones are found at operation, may also indicate excessive blood destruction while considerations of family history and race are obviously important. It will be apparent, however, from later discussion that the modes of presentation of haemolytic disease are several and that diagnosis depends to some extent on the clinical setting but more so on the analysis of a number of investigations, each of which gives particular information and has a distinct purpose. There are first, routine and simple tests which show the indirect results of haemolysis, namely changes in bile pigment metabolism due to increased bilirubin production from excessive red cell destruction, and the changes in peripheral blood and bone marrow which reflect compensatory erythropoiesis. If these tests when considered together do not permit a confident diagnosis of haemolytic disease, then it is likely that the rate of blood destruction is not greatly increased and direct measurement of red cell survival will be necessary for full evaluation of the case.

Having/

Having determined that a haemolytic process is present, it must then be established whether the condition is due to an intrinsic red cell defect or to some extracorporeal mechanism and to determine precisely which condition is present. Some information may have been gained regarding defects in the red cell from the routine examination of their morphological characteristics in films of the peripheral blood. This will be so when the defect is pronounced and applies particularly to spherocytes, elliptocytes, target cells and sickle cells. In general, more sophisticated examination of the erythrocytes is required. This involves tests of fragility or susceptibility to destruction; electrophoresis of haemoglobin; measurements of glucose-6-phosphate dehydrogenase deficiency, either through the production of Heinz bodies on exposure of the corpuscles to aromatic nitrogen compounds (Fertman and Fertman 1955), or through the glutathione stability test, a measurement of reduced glutathione levels in red cells before and after incubation with acetylphenylhydrazine (Beutler 1957). Finally the acid serum test of Ham (Ham 1939) may be required; this with suitable control (Dacie 1949) is diagnostic of paroxysmal nocturnal haemoglobinuria.

Search for extracorporeal factors entails in practice examination and investigation of the patient for diseases that are known to be associated with excessive blood destruction and examination of the patient's blood for red cell/

cell antibodies; recognition of the former since they are mainly diseases of the reticulo-endothelial system and connective tissue can usually be achieved by study of the blood and bone marrow, biopsy of certain tissues (e.g. marrow, lymph node and liver) and screening for disseminated lupus erythematosus particularly using LE cell preparations. Serological studies begin with Coombs' test but then follow complex procedures for characterisation of red cell antibody to which reference will be made.

Not all the methods of investigation of haemolytic disease have been required in this thesis. The techniques employed will be described in five sections:-

- (i) Routine tests of blood, urine and bone marrow; procedures to exclude underlying disease,
- (ii) Red cell fragility tests,
- (iii) Serological studies,
- (iv) Red cell survival measurements using chromium-51. Since these have a major role in the thesis they will be discussed in detail.
- (v) Ancillary investigations that are more concerned with the effects of haemolytic disease than with diagnosis.

2. 3. Routine tests.

The tests used in the initial assessment of patients that are referred to in this section have been undertaken in the routine haematological, biochemical and pathological departments of the Edinburgh hospitals and the Memorial Hospital, New York.

Peripheral blood

Haemoglobin estimation and reticulocyte counts have always been undertaken since these have been found to be the most useful single measurements, both for diagnostic purposes and for the assessment of a patient's progress. Haemoglobin levels will be referred to as a percentage; $100\% = 14.8 \text{ g. Hb./100 ml.}$ Reticulocyte counts will be expressed as a percentage of the red cell count; the normal value has been accepted as less than 2% (Dacie 1956). Films of peripheral blood have been made in all patients; frequently spherocytosis accompanying reticulocytosis has been the basis for suspecting a haemolytic disorder; examination of blood films has also been essential to the exclusion of leukaemia. Since the absolute indices (mean corpuscular volume, M.C.V.; mean haemoglobin concentration, M.C.H.C.) and the white cell count have not been found to contribute to the diagnosis or to the management of a patient, these values are not available for every patient. The common occurrence of macrocytosis that was known many years ago (Dameshek and Schwartz 1940) has been confirmed.

The/

The reason for a raised mean corpuscular volume is uncertain; it may be partly due to the presence of reticulocytes but this cannot be the whole explanation (Dacie 1960), and it seems possible that the macronormoblasts found in the marrow in haemolytic disease (Dacie and White 1949) might well be the source of abnormally large corpuscles.

Bone marrow

The marrow has been examined in most patients; aspiration of the medullary cavity in the sternum or ilium has always been attempted first and if unsuccessful (e.g. in the patients with myelofibrosis) marrow trephine has been undertaken. Study of the marrow has not been used to confirm the presence of a haemolytic process but rather to exclude leukaemia, reticulosis and myelofibrosis. More recently, since the reports of Crosby and Sacks (1949), Chanarin, Dacie and Mollin (1959) and Delamore, Richmond and Davies (1961), there has been interest to exclude megaloblastic erythropoiesis.

Bile pigments

The work of Lathe and his colleagues (Cole and Lathe 1954, Billing and Lathe 1958) has led to an understanding of the changes in bile pigment metabolism that occur in haemolytic disease. Their chief observation is that bilirubin from haemoglobin catabolism (prehepatic bilirubin, haemobilirubin, unconjugated bilirubin) is converted into water soluble forms in the liver by conjugation with hydrophilic compounds/

compounds of which glucuronic acid is the most important. This difference between prehepatic bilirubin, which is bound to protein and cholesterol in the plasma, and post hepatic bilirubin (hepatobilirubin, conjugated bilirubin), as is regurgitated into the plasma in obstructive jaundice, has explained the direct and indirect Van den Bergh reactions and also the mechanism of production of "acholuric jaundice".

There are three measurements by which bile pigment metabolism may be sampled easily; the bilirubin level in the plasma and the level of urobilinogen in the urine and in the faeces. There are, however, a number of factors that govern the quantity of bilirubin produced; the chief of these are the rate of red cell destruction leading to catabolism of haemoglobin and the red cell mass. Other factors govern the actual level of bilirubin in the plasma and the amount of urobilinogen that is excreted; the chief of these, other than the amount of bilirubin that is produced, is the capacity of the liver to deal with it. Quantitative measurements of urobilinogen were formerly considered to be useful indices of haemoglobin breakdown and the "haemolytic index" (Miller, Singer and Dameshek 1942) was an attempt to relate the daily output of urobilinogen in the faeces to the total mass of circulating haemoglobin. However, in addition to the influences on bile pigments already mentioned there are a number of other reasons why such/

such techniques are not of value and why no attempt has been made to employ them in the present study. First, there is the uncertainty regarding the degree of reabsorption of urobilinogen from the bowel. Second, it has been shown from studies with nitrogen-15 labelled glycine that a significant proportion of urobilinogen, probably in the region of 15% in normal individuals, and more in haemolytic disorders, is derived from sources other than haemoglobin within mature circulating erythrocytes (London, West, Shemin and Pittenberg 1950); a number of porphyrins of endogenous origin, such as myoglobin, probably contribute to the total urobilinogen (Lemberg and Legge 1949, Schmid 1959); it is also suggested (Bingold 1949) that there may be oxidation products of haem, such as dipyrroles, which are not included in the measurement of urobilinogen. These different findings undoubtedly help to explain why a number of investigators (Hagen and MacDonald 1954, Crosby 1955, Baldini and di Pietrantonio 1957) have failed to recover the expected amount of urobilinogen from patients with various haemolytic disorders.

On the few occasions that the direct and indirect reacting bilirubin of the Van den Bergh reaction has been measured it has not been found to make a significant diagnostic contribution.

Taking liver function into account it has been found sufficient/

sufficient, both for diagnosis and for following the progress of most patients, to estimate the serum level of bilirubin and qualitatively, using Ehrlich's aldehyde reagent, to assess the degree of urobilinogenuria.

Presumably because of the large capacity of the liver to excrete bile (Billing and Lathe consider that the healthy liver could excrete all the bilirubin normally contained in the plasma in ten to twelve hours), the serum bilirubin is seldom high in haemolytic disease; Dacie (1960) states that the level may be normal, is usually in the range 1 - 3 mg. and rarely exceeds 5 mg.

Haemoglobin breakdown products in severe haemolysis

When haemolysis is very severe and because much of the red cell destruction is intra-vascular, free haemoglobin accumulates in the plasma. It is now known that the haptoglobins have the capacity for combining with haemoglobin and are probably the main pathway by which it is cleared from the blood (Jayle and Boussier 1955). Smithies (1955a, 1955b) showed that the haptoglobins were saturated by haemoglobin in a concentration of the order of 125 mg./100 ml.; Laurell and Nyman (1957) found that the level of saturation varied in normal subjects in the range 50 - 140 mg./100 ml., and when saturation occurred excess of haemoglobin that had been given intravenously caused haemoglobinuria. Haemoglobinaemia, methaemalbuminaemia and haemoglobinuria, the various results of rapid haemoglobin degradation/

degradation have not been looked for routinely but will be discussed in the two patients with autoimmune haemolytic anaemia in whom their estimation was relevant.

Exclusion of underlying disease

In addition to the routine examination of blood, bone marrow and urine that was undertaken for signs of increased erythropoiesis and of increased haemoglobin catabolism, it was in some patients necessary to exclude underlying disease by lymph gland biopsy, liver biopsy and examination of the peripheral blood for the LE cell phenomenon. Reference will be made to these procedures where applicable. For the demonstration of LE cells the methods described by Hargraves (1954) and Snapper and Nathan (1955) have been used.

2. 4. Red cell fragility tests

There is no single satisfactory test which correlates susceptibility of red cells to destruction in the circulation with a measurable abnormality in the laboratory. This would be expected from the different mechanisms of red cell destruction in each disease; for example, when the patient has a high titre of cold antibodies (p. 65), it is believed that agglutination of the patient's corpuscles occurs in the extremities, particularly in a cold environment, and that the agglomerates of red cells are trapped and traumatised in their further passage through the circulation; in sickle cell disease it is likely to be the shape/

shape of the cell that will cause damage from mechanical factors; in glucose-6-phosphate dehydrogenase deficiency it is the age of the cell that is important and then only if a particular aromatic compound is in the surrounding medium.

Mechanical fragility

Although not the first to be devised, the most rational type of in vitro test offers some form of mechanical trauma to mimic the effect of the circulation. Meltzer and Welch (1884) drew attention to the destruction of erythrocytes by physical factors but it was not until the work of Dameshek and Miller (1943) and Shen, Castle and Fleming (1944) that mechanical fragility tests suitable for clinical application were devised. The early investigations showed that mechanical fragility paralleled osmotic fragility (see below) and that agglutinated erythrocytes and sickled erythrocytes were also susceptible to mechanical destruction. Schaub and Maier (1956) have reported the widest experience and found mechanical fragility tests to be of value in doubtful cases of hereditary spherocytosis, showing a positive result when the osmotic fragility test was in the normal range.

Most methods have depended on the agitation of uncoagulated blood with glass beads. The results obtained in a small series of patients, using a modification of the method described by Dacie (1956) are given in Table 2.

The/

TABLE 2

COMPARISON OF FRAGILITY OF RED CORPUSCLES IN
DIFFERENT DISEASES TO MECHANICAL TRAUMA

Condition of Subjects	No.	Percentage Haemolysis
Normal	6	2.8 - 5.6
Congenital spherocytosis	3	5.1 - 11.3
Elliptocytosis	1	5.2
Thalassemia minor	1	3.6
Autoimmune haemolytic anaemia	2	4.6 - 6.0
Symptomatic non-immune haemolytic anaemia	2	1.8 - 5.1

The patients with autoimmune haemolytic anaemia and symptomatic non-immune haemolytic anaemia had a severe haemolytic process; these results were not felt to contribute to the present study and mechanical fragility tests were abandoned.

Osmotic fragility

According to Wintrobe (1961), it was Hamburger (1883) who first proposed a fragility test which measured haemolysis of red cells in hypotonic salt solutions. Chauffard (1907) introduced the present type of test and considered that the wide "resistance span" in haemolytic jaundice was an expression of heterogeneity of cell population in respect of diameter. Following the work of Haden (1934), who correlated increased susceptibility to haemolysis with a falling diameter-thickness ratio, Castle and Daland (1937) in extensive studies concluded that differences in the susceptibility of erythrocytes to haemolysis with hypotonic sodium chloride were due to differences in form and not to differences in osmotic behaviour. Direct microscopic observations convinced them that the erythrocytes assumed a spherical shape before haemolysis and the more susceptible the erythrocyte to "hypotonic haemolysis" the less hypotonic the plasma necessary to cause the production of a spherical form. The paper of Hunter (1940) probably introduced the present quantitative methods for determining "osmotic fragility"/

fragility" of erythrocytes by increasing haemolysis photo-colorimetrically. Despite the general acceptance of these pioneer observations, Sharpsteen (1955) showed convincingly that the intracellular protein osmotic pressure and the tensile strength of corpuscular membranes was more important in determining red cell fragility than the conformation of the cell.

Numerous reports now indicate that increases of fragility are seen in all haemolytic disorders that are associated with spheroidicity of the corpuscles. Hence (Dacie 1960) it is found in almost all patients with congenital spherocytosis, in most patients with autoimmune haemolytic anaemia, in some patients with chemically induced haemolysis and after extensive burns. Dacie (1943) pointed out the "tailed" type of curve that is characteristic of congenital spherocytosis, and Bolton (1949) and Emerson, Shen, Ham, Fleming and Castle (1956) demonstrated the disappearance of the population of very sensitive cells, which account for the "tail", after splenectomy in this condition. Increased fragility in autoimmune haemolytic anaemia has been known since the work of Dameshek and Schwartz (1938). In elliptocytosis, when haemolysis is present, osmotic fragility may or may not be increased (Dacie 1960). In conditions where the red corpuscles have an increased diameter-thickness ratio, as seen in the target cells (Barrett 1938) of thalassaemia and chronic liver disease, there is seen to be resistance/

resistance to osmotic haemolysis. In many anaemias, notably iron deficiency and pernicious anaemia (Cassells 1938), the red cells are less fragile than in health, the fragility improving as the cells become normal. Simon and Topper (1957) and Pranker (1958) have found, using radioactive iron as a red cell label, that young red cells are less fragile than old red cells.

Method used for measuring osmotic fragility.

The method used for determining osmotic fragility in the present studies was modified from that described by Wintrobe (1956). A stock solution with the osmotic activity of 10% sodium chloride was prepared by dissolving 18 g. sodium chloride, 2.731 g. dibasic sodium phosphate and 0.486 g. monobasic sodium phosphate ($2H_2O$) in 200 ml. of distilled water. This was kept in a stoppered flask at 4° C. and was replaced at the first hint of bacterial contamination. Using a 1% working solution, 10 ml. of the following dilutions of sodium chloride (expressed as percentage) was prepared in matched test tubes:- 0.9, 0.85, 0.8, 0.75, 0.70, 0.65, 0.60, 0.55, 0.52, 0.50, 0.48, 0.46, 0.44, 0.42, 0.40, 0.38, 0.35, 0.30, 0.25, 0.20, 0.10.

Ten ml. of distilled water was added to a final tube and this tube was used to indicate 100% haemolysis.

To each tube was added 0.05 ml. blood which had been anticoagulated by sequestrene and previously oxygenated by frequent rotation in the tube (Creed 1938) under test;
each/

each test was done in duplicate and on each occasion that osmotic fragility tests were undertaken the blood of a normal individual was tested as a control.

The test tubes, after thorough mixing, were incubated for thirty minutes at 37° C. They were then centrifuged and the amount of haemoglobin in the supernatant fluid in each tube was determined in an EEL (Evans Electroselenium Limited) colorimeter, previously calibrated for different dilutions of haemoglobin. The data were then plotted graphically with the percentage haemolysis as ordinate and the percentage concentration of saline as abscissa.

Results of osmotic fragility tests in normal subjects and an illustration of abnormal results.

Fig. 1 shows the results obtained in twenty-six normal subjects, in various haemolytic disorders and in other disorders of the blood. The observations are typical of those obtained by others. The "tail" of very fragile cells seen in some patients with congenital spherocytosis is illustrated, as is the normal fragility of cells from a patient with symptomatic non-immune haemolytic anaemia and the comparative resistance of macrocytic cells, iron deficient cells and cells from a patient with thalassaemia minor. Charting the increments of haemolysis between successive concentrations of sodium chloride as suggested by Momigliano, Levi and Bairati (1935) and practised by others, particularly Bolton (1949), emphasises deviations from the normal/

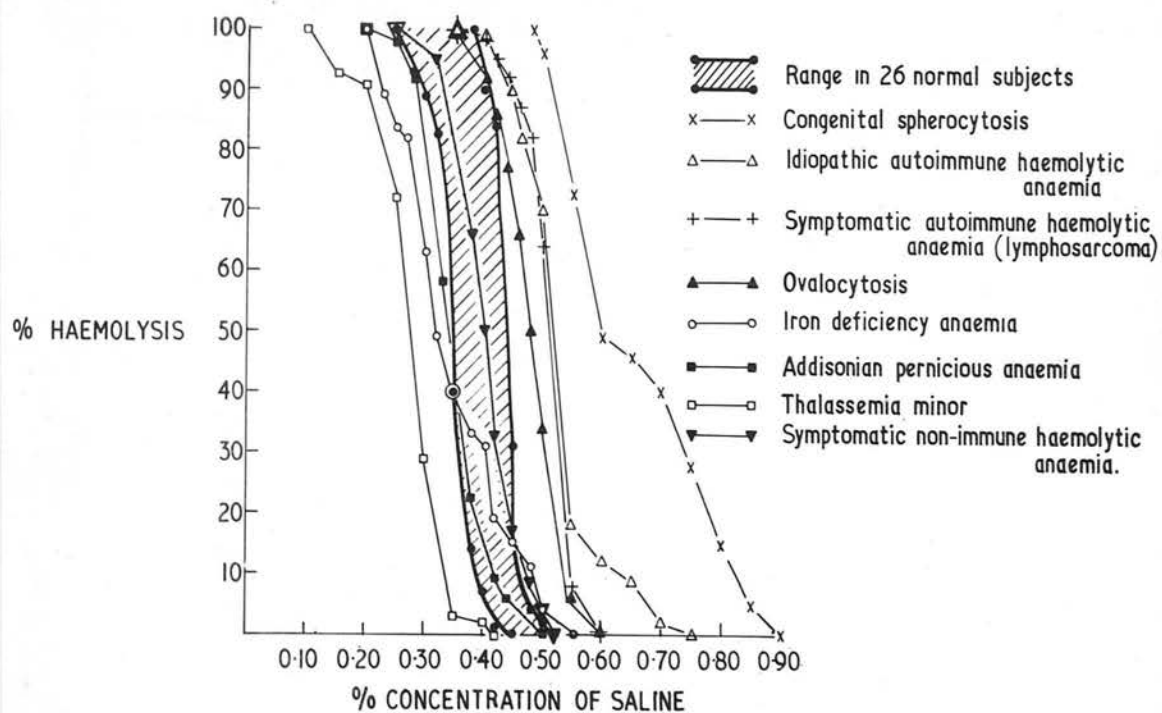


Fig. 1

The lysis of red blood cells in hypotonic solutions of saline (osmotic fragility) in normal subjects and in various haematological disorders.

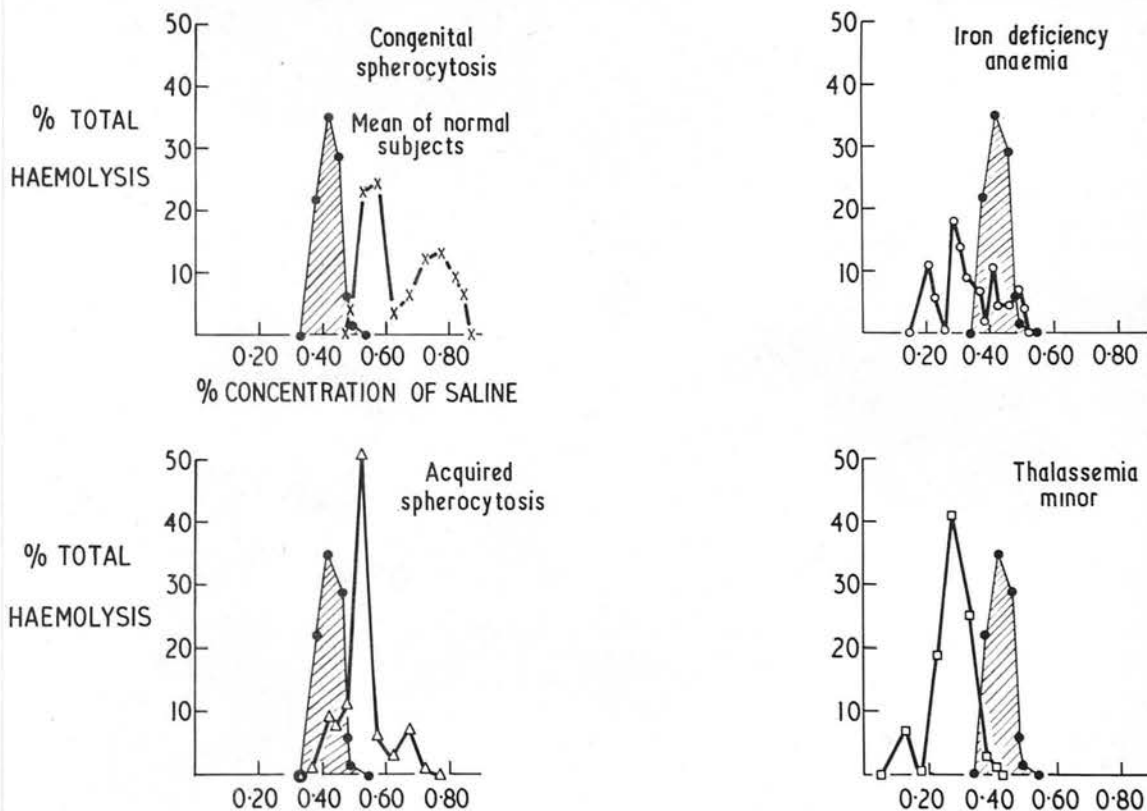


Fig. 2

The increments of haemolysis between successive dilutions of saline in the osmotic fragility test. This method of recording the data illustrates cell populations of differing fragility and emphasises "shifts" to the left or the right of the normal range.

The results in actual subjects are given in Table 3.

normal as is illustrated in Fig.2. This procedure helps to indicate populations of cells of different susceptibilities to osmotic haemolysis but does not otherwise add to the interpretation of the data.

Various workers, notably Emerson, Shen and Castle (1944), Young (1947) and Dacie, Mollison, Richardson, Selwyn and Shapiro (1953), have used the observation that the red cells of congenital spherocytosis undergo greater increase of osmotic fragility than do normal cells after the blood of the patient has been incubated under sterile conditions for twenty-four hours at 37° C. before undertaking the test.

Young, Izzo and Platzer (1951) in studies of seventeen patients with congenital spherocytosis showed how this procedure increased the chance of diagnosis in patients with mild degree of red cell abnormality. Results in three patients with congenital spherocytosis, in two of whom osmotic fragility was not markedly abnormal and in whom the presence of spherocytes in blood films was doubtful, is shown in Fig.3.

In referring to results of osmotic fragility tests, two measurements will be described; first the concentrations of saline where haemolysis begins and haemolysis is complete and second the concentration of saline (calculated from a graph of the data) at which 50% of the patient's erythrocytes have been haemolysed.

The results in normal subjects are given in Table 3.

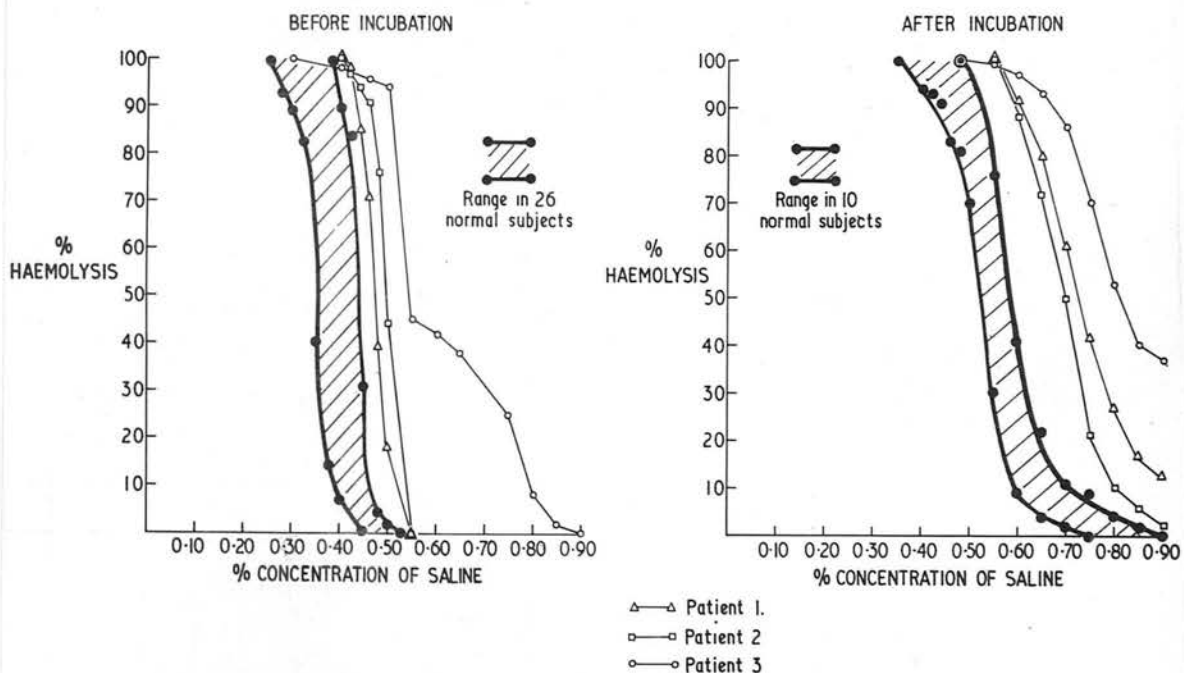


Fig. 3

The effect of incubation of blood for 24 hours on the osmotic fragility of red cells. This technique is useful in distinguishing patients with mild spherocytosis (Patients 1 and 2), since the deviation from normal after incubation is increased, compared with that obtained in the standard test.

TABLE 3

RESULTS OF OSMOTIC FRAGILITY TESTS IN NORMAL SUBJECTS

A. Standard procedure

(26 individuals)

% concentration of sodium
chloride

	Range	Mean
Onset of haemolysis	0.52 - 0.45	0.490
Completion of haemolysis	0.38 - 0.25	0.305
50% haemolysis	0.440 - 0.345	0.395

B. Incubation procedure

- see text

(10 individuals)

Onset of haemolysis	0.90 - 0.75	0.835
Completion of haemolysis	0.48 - 0.35	0.405
50% haemolysis	0.585 - 0.520	0.560

2. 5. Serological tests.

Tests for the detection of red cell antibody have been used in the evaluation of all but five of the patients that have been studied. They have been undertaken for the Edinburgh patients by the staff of the Blood Transfusion Service for the South-Eastern Region of Scotland by courtesy of Dr. R. A. Cummings, and for the American patients by the staff of the Haematology Department, Memorial Center for Cancer and Allied Diseases, New York, by courtesy of Dr. Allyn B. Ley.

To make for clarity in later discussion of investigations, a brief outline of the serological procedures that have been employed follows. (Since each laboratory uses its own modification of the various techniques, only the originator of a test will be referred to where relevant.)

(i) On receipt of a sample of clotted blood from the patient, the cells have been separated from the serum.

(ii) The cells were typed for ABO and Rhesus D blood groups; genotyping followed where indicated.

(iii) The patient's cells were then incubated at 37° C. with Coombs' antiserum (Coombs, Mourant and Race 1945)

[antihuman globulin serum prepared by immunising a rabbit] usually at two specified dilutions of serum, one of which is likely to detect antibodies of the Y globulin type (in the region of 1/40), and the other likely to detect antibodies of/

of the non- γ globulin type (in the region of 1/10). This is the "Direct Coombs' test" which should recognise that the patient's red cells are coated with globulin.

(iv) A series of tests were applied to the patient's serum. Dilutions of serum were mixed with red cells in saline suspension and albumen suspension (Diamond and Denton 1945); serum was added to cells previously treated with a proteolytic enzyme, e.g. papain (Morton and Pickles 1947, 1951); it was incubated with compatible cells in an attempt to sensitise them, after which these cells were incubated with Coombs' antiglobulin serum (Indirect Coombs' test). All these tests for free antibody in the patient's serum utilised a panel of red cells selected to include the common blood group systems (ABO, Rh, MNS, P, Lewis, Kell, Duffy and on occasions Lutheran and Kidd). The tests were done at two levels of temperature, 40° C. and at 37° C.

If the screening tests in (iii) and (iv) were negative it was considered to be virtually certain that no red cell antibody was present. If positive, it was possible to state whether there appeared to be one antibody or a multiplicity of antibodies, whether complete or incomplete, whether warm or cold (indicating the in vitro temperature at which they held greatest activity) i.e. the thermal range, and whether the antibody had shown any specificity for the panel of blood cells used.

(v)/

(v) More specialised tests followed. The first was usually the "Y globulin neutralisation test" (Coombs and Mourant 1947, Dacie 1951), in which the Direct Coombs' test was repeated in the presence of dilutions of Y globulin. If complete neutralisation occurred the antibody was believed to be a Y globulin and therefore also almost certainly of the warm type; if no neutralisation was seen the antibody was referred to as a non-Y globulin (and was almost always of the cold type); partial neutralisation usually indicated that a mixture of antibodies was present.

Then followed an attempt to elute the coating antibody from sensitised cells, usually by heating them for ten minutes at 56° C. (Kidd 1949, Weiner 1957). The eluate was treated in the same way as the patient's serum (see (iv) above) to decide if the coating antibody was of the same nature as any free serum antibody that may have been found.

Specificity was decided by incubating the patient's antibody in serum or in red cell eluate with a narrowing panel of cells, using homozygous cells in respect of blood groups where necessary. Where several antibodies are present, the character of each can only be determined by laborious absorption tests in which each antibody is removed from the serum or red cell eluate in turn, and this was seldom considered to be necessary.

(vi)/

(vi) Finally it was of value, not in determining the severity of a haemolytic process, but in following the progress of an individual patient to perform a quantitative Coombs' test titration (Wiener and Gordon 1953). This was achieved by setting up the Direct Coombs' test using serial dilutions of antiglobulin serum, e.g. from 1/10 to 1/1250. The result for each dilution was charted depending on the strength of agglutination: -ve, $\frac{+}{2}$, +, ++, +++, +++++.

2. 6. Studies using radioactive chromium-51 as a red cell label.

Other techniques for red cell survival measurement

The introduction of the differential agglutination technique of Ashby (1919) for the measurement of red cell survival has been discussed on page 13. The method was improved over the years, particularly when MN blood groups (Landsteiner, Levine and Janes 1928) and the Rh blood groups and antisera (Mollison and Young 1942, Wiener 1942) were utilised; Hurley and Weisman (1954) added a further refinement by developing the use of potent haemolytic antisera to obviate errors due to the trapping of cells in agglutinates (e.g. transfused O cells might be trapped in agglutinates of the recipients' A or B cells).

The Ashby technique has always had the limitations that study of an individual was governed by considerations of/

of blood group, that it was necessary to begin the measurement with the transfusion of two to three pints of blood, but most important, by the nature of the technique it was never possible to study the survival of a patient's own cells in his own circulation.

Because of the obvious advantage of using isotopes as red cell labels, a number have been tried. Nitrogen-15 and carbon-14 have been administered in aminoacids, particularly glycine, for incorporation in haemoglobin (Shemin and Rittenburg 1946, Bale, Yuile, Delavergne, Miller and Whipple 1949, Berlin, Lawrence and Lee 1951), but both of these isotopes are difficult to use for routine purposes; the first is a stable isotope and the second emits only low energy β radiation. Radioactive iron has been employed extensively but the conservation of iron derived from the catabolism of haemoglobin of senescent red cells for re-utilisation in haemoglobin synthesis makes difficult the interpretation of changes in the specific activity of red cells (i.e. the concentration of radioactivity per unit volume of red cells) as a measure of red cell survival. One or other of the two isotopes of iron, ^{55}Fe and ^{59}Fe , may be used either to label the cells of an individual who is later going to donate blood to the patient under study (Ross and Chapin 1943, Gibson, Aub, Evans, Peacock, Irvine and Sack 1947), or the patient's plasma may be tagged with radioactive iron which reappears in newly formed red cells increasingly/

increasing over the next ten days. In normal subjects and some patients with haemolytic disease, where compensatory erythropoiesis is not active, the approximate survival time of the labelled red cells is seen as a small dip in the graph of radioactivity in the patient's circulation against time (Huff and Judd 1956, Berlin, Beeckmans, Elmlinger and Lawrence 1957). Radioactive phosphorus in the form of sodium orthophosphate is taken up by red corpuscles very readily and it is useful in measurements of red cell mass; it escapes, however, from the cells at the rate of 10-12% per hour thereafter and is valueless in studies of red cell life. Phosphorus-32 has, however, been used in diisopropyl fluorophosphonate (DF^{32}P); this compound inhibits cholinesterase activity in the red cell with the result that the phosphorus label is fixed; using this effect measurements of red cell survival have been achieved (Cohen and Warringa 1954).

The introduction of chromium-51 as a red cell label

When Gray and Sterling (1950) discovered that trace amounts of chromium were normally present in red blood cells and that sodium chromate containing radioactive chromium-51 became fixed to the corpuscles of dogs, a new and valuable technique for measuring red cell life span became available. Chromium-51 has a radioactive half life of 27.8 days and a biological half life of 110 days. It decays by K electron capture, 8% of disintegrations being associated with the emission of X-rays of energy 0.32 MeV. In principle, for studies/

for studies of cell survival the red cells (either the patient's own cells or donor cells) are labelled in vitro with radioactive sodium chromate ($\text{Na}_2^{51}\text{CrO}_4$), injected into the circulation of the recipient, and the rate of change of ^{51}Cr concentration per unit volume of red cells is determined. Experiments have shown that red cells mixed with acid citrate dextrose mixture take up chromium-51 more readily than do cells from heparinised blood (Necheles, Weinstein and Le Roy 1953, Mollison and Veall 1955); the uptake of chromium on incubation of red cells with radioactive sodium chromate at room temperature is almost complete after thirty minutes and chromium remaining in the supernatant at this stage can be almost completely removed by washing the cells twice or three times and re-suspending them in saline. The washing of chromium-labelled cells up to twenty-five times does not affect their subsequent survival (Necheles, Weinstein and Le Roy 1953, Hughes-Jones and Mollison 1956, O'Brien 1958). Examination of the effect of concentrations of chromium certainly up to $10\text{ }\mu\text{g.}$ per ml. of red cells also does not affect the survival data; according to Hughes-Jones and Mollison (1956) concentrations of the order of $30\text{--}40\text{ }\mu\text{g.}$ per ml. of red cells induce methaemoglobin production.

Hexavalent chromium-51 (as in sodium chromate) has the unique advantage over all other red cell labels which have been used to date in that there is no evidence of re-utilisation/

utilisation of chromium released from labelled cells; there is no transfer of the isotopic label to red cells from circulating ^{51}Cr -tagged haemoglobin, haemolysed red cells or from incompatible cells (Jandl, Greenberg, Yonemoto and Castle 1956), and it seems likely that this property results from the conversion of anionic hexavalent chromium to the cationic trivalent form once the chromium has entered the red cell; the latter is known to bind firmly with protein but will not penetrate the corpuscular membrane.

The use of chromium-51 for measurements of red cell survival was largely developed by the studies of Ebaugh, Emerson and Ross (1953), Necheles, Weinstein and Le Roy (1953), Read, Wilson and Gardner (1954) and Mollison and Veall (1955). Two factors emerged from the early work that greatly influence the interpretation of red cell survival data obtained by this method and which have not yet been fully explained. Firstly, the graph of the ^{51}Cr content of red blood cells injected into a normal subject as a function of time is curvilinear and not straight; in comparative studies using the ^{51}Cr method and the Ashby method of differential agglutination, chromium disappears from the circulation more rapidly than the red cells, and this is presumed to be due to the elution of the metal from the cells. Secondly, it is clear that the rate of elution of radioactive chromium is not the same throughout the period/

period of a survival measurement; there are at least two components (Mollison and Veall 1955), a rapid component in the first few hours when 5-10% of the chromium label is eliminated with a half period of approximately 1.5 days, and a slower component affecting the remainder which is calculated to be 54.2 days.

The interpretation of red cell survival data obtained with chromium-51.

Problems of elution of chromium-51, limitations as to the dose and specific activity of chromium that can be used and the half life of the isotope have led to a number of methods of analysis of the data. All the methods yield only comparative information; perhaps the most acceptable is to follow the disappearance of radioactivity from a patient's circulation to the point of extinction, but this might mean continuing the survival measurement for more than 100 days and such duration of study is seldom practicable in clinical investigation.

A common method in current use is to plot the specific activity of the red cells as a function of time and calculate the time required for the injected radioactivity to decrease in the circulation by 50%. This time has been referred to as the $T_{1/2}^{Cr}$.

Table 4 gives a summary of the results of red cell survival measurements using chromium-51 in normal subjects compiled by Berlin, Waldmann and Weissman (1959).

It/

TABLE 4

ANALYSIS OF PUBLISHED RESULTS OF RED CELL SURVIVAL
MEASUREMENTS IN NORMAL SUBJECTS USING CHROMIUM-51.

(from Berlin, Waldmann and Weissman 1959)

PARAMETER	NO. OF SUBJECTS	RANGE	MEAN
$T_{1/2}Cr$ (days)	163	25 - 40	29
Extinction of radioactivity in circulation (time in days)	37	108 - 120	113
Calculated rate of elution of ^{51}Cr from labelled cells (per cent per day)	22	0.57 - 1.17	0.93

It will be seen that there is considerable variation in the $T_{1/2}^{51}\text{Cr}$ obtained by different investigators; this is discussed by Mollison (1961) who presents evidence that the scatter of data is due to different rates of elution of chromium consequent on differences in technical procedure.

The question of the practical interpretation of the ^{51}Cr method has been discussed by Hughes-Jones and Mollison (1956). It has first to be decided if any correction for elution of chromium should be applied. There is no problem when the $T_{1/2}^{51}\text{Cr}$ falls within the range of values obtained by a particular investigator for his series of control subjects, since such results can be taken as being normal. Similarly, there is no problem when red cell destruction is considerably increased because the question of a rapid component of chromium elution and the exact method of correction for chromium elution becomes unimportant. The difficulty arises in patients where red cell life span is reduced, but not grossly so, because whether the chromium-51 results are corrected for chromium elution or not, their interpretation depends on the assumption that the rate of elution is the same in the disease that is being studied as in normal subjects. Cline and Berlin (1963) have recently presented evidence to suggest that the rate of elution may indeed be slightly more rapid in haematological disorders than in health. In thirty-eight patients with a wide range/

range of conditions, they obtained an average elution rate of ^{51}Cr of 1.29 per cent per day.

It is for these reasons that several methods have been recommended for deducing true red cell survival from data obtained using chromium-51. For example, it is suggested by Hughes-Jones and Mollison (1956) that true survival can be derived from the expression:-

$$\frac{\text{Nt } ^{51}\text{Cr observed}}{\text{Nt } ^{51}\text{Cr normal}} \times \frac{(110 - t)}{(110)}$$

where Nt ^{51}Cr = percentage injected ^{51}Cr remaining in the circulation on day t. This assumes a mean cell life in the normal of 110 days, that the range of elution rates in normals is small and that the elution rate in disease states is the same as in the normal. Subsequently the corrected estimates are plotted and the mean cell life is calculated as described by Dornhorst (1951). There is evidence that this method over-corrects when applied to abnormal cells, (Mollison 1959), and a more widely used treatment of data (Ebaugh, Emerson and Ross 1953) in haemolytic disorders is to assume a steady rate of elution of chromium with a half period of approximately seventy days.

The use of body surface measurements of radioactivity.

Although the radiation from chromium-51 is of low energy and is highly absorbed by tissues, it has been possible, by making measurements over the surface of the body, to follow the distribution of chromium-51 labelled erythrocytes/

erythrocytes during the course of red cell survival measurements. Hughes-Jones and Szur (1957) showed that in normal subjects the only significant collections of radioactivity were found over the heart, over the liver and over the spleen, presumably in consequence of the blood flow through these areas. Korst, Clatanoff and Schilling (1955), appreciating the possible role of this in the management of haemolytic disease, found that the relative accumulation of radioactivity in the liver and spleen was very variable, and it was only in two patients out of three who showed a higher uptake of radioactivity in the spleen compared with the liver that splenectomy was beneficial. Jandl, Greenberg, Yonemoto and Castle (1956) in observations in various haemolytic disorders tried to quantitate their data by using the "spleen sequestration index" which was the percentage increment in the spleen/praecordium ratio of radioactivity between the start of the survival study and the $T_{1/2}^{51}\text{Cr}$. These authors offered a tentative grading of intensity of splenic sequestration on the basis of this index and suggested that less than 30 was subnormal, 30-60 normal, 60-100 mild to moderate and more than 100 moderate to severe. In the work of Hughes-Jones and Szur (1957), an attempt was made to correct the surface measurements for radioactivity due to perfusing blood and they also showed that when there was a high uptake of ^{51}Cr in spleen tissue this was an indication of the likely value of splenectomy. Goldberg (1960)/

(1960), using the spleen/liver ratio of radioactivity at $T_{1/2}Cr$ as originally suggested by Korst, Clatanoff and Schilling (1955), found that in acquired haemolytic anaemia when this ratio exceeded 2.4 remission followed removal of the spleen.

These measurements of surface radioactivity have great value in helping to understand the pathogenesis of different forms of haemolytic disease; they also have a possible place in evaluating the role of the spleen and in trying to establish which patients are likely to benefit from splenectomy. The number of methods advanced for analysing the data is an indication of the difficulties of interpretation that have been encountered.

Methods used in the present study.

Chromium-51 was obtained in 2 millicurie amounts from The Radiochemical Centre, Amersham. In the Memorial Hospital, New York, supplies were obtained from Abbot Laboratories Ltd. The specific activity was always in the range 50-100 $\mu c./\mu g$. On receipt, the consignment was diluted to 10 ml. with normal saline to facilitate dispensing as the radioactivity decayed.

The method of labelling erythrocytes was modified from that described by Mollison and Veall (1955). Ten to 20 ml. of blood (either the patient's own blood or, where applicable, donor blood that was less than forty-eight hours old) were added to a bottle of 25 ml. capacity and mixed with

3-5 ml. acid citrate dextrose solution or with six drops of heparin (5,000 units/ml.). Fifty to 100 μ c. of chromium-51 were then mixed with the blood, and the bottle was incubated at room temperature for at least forty-five minutes. After incubation, the cells were washed twice with normal saline and reconstituted to a volume of 15 ml. A precise amount, usually 12 ml., was injected intravenously into the patient and 1 ml. retained as a standard, if blood volume measurements were desired; otherwise and in most cases, the whole of the red cell suspension was given.

Samples of blood were then withdrawn from the patient after one hour, twice in the next four days, and thereafter every three to seven days. The study was always continued for twenty days unless the amount of radioactivity in the blood had fallen to levels of 30-40% of that injected earlier than this.

Blood was collected in tubes containing approximately 0.5 g. sodium oxalate and 0.5 g. saponin. The radioactivity in these serial samples was then measured with orthodox equipment which combined a well type scintillation counter and a scaler (Ecko, Panax or Nuclear-Chicago), appropriate correction being made for background radiation. To avoid the need for correction for radioactive decay the blood samples from a particular patient were always counted together.

One/

One hour after the radioactive labelled blood had been injected and thereafter, at intervals similar to that used for venous sampling, measurements of radioactivity over the surface of the body were made in selected patients using a directional scintillation counter (Ecko, Panax or Nuclear-Chicago) and a scaler. After preliminary studies the patient was always examined in the supine posture and the following positions at contact with the skin were used for the directional counter; for the heart, the fourth intercostal space, one inch from the left sternal edge, for the liver, the right midclavicular line, one inch above the costal margin, and for the spleen, the left mid axillary line at the tenth rib, the counter being in a horizontal plane. For analysis of some of these body surface data, the radioactivity in a ^{51}Cr standard, prepared as described by Hughes-Jones and Mollison (1956), was measured at contact with the counter.

Measurements of red cell survival in normal subjects with illustrations from different disease states.

The data for survival of the individual's own red cells obtained in nine normal subjects are illustrated in Fig. 4. The radioactivity in 102 samples of blood are plotted and the study in each individual has been followed for thirty or more days. As has been reported for all other normal data, the specific activity of injected chromium-51 as a function/



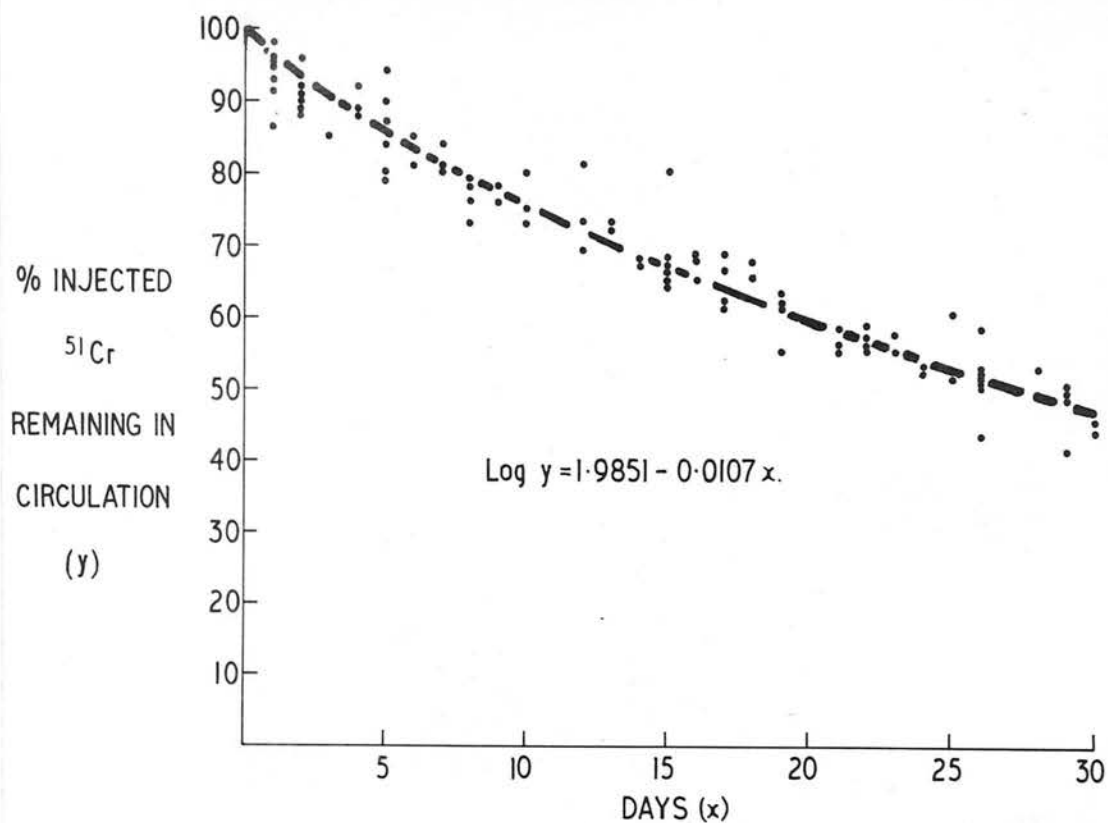


Fig. 4

Red cell survival measurements in 9 normal subjects using chromium-51 as a red cell label. The data are uncorrected for chromium elution. The bold interrupted line represents the regression of the log. percentage chromium-51 remaining in the circulation on time and will be repeated in subsequent figures for reference.

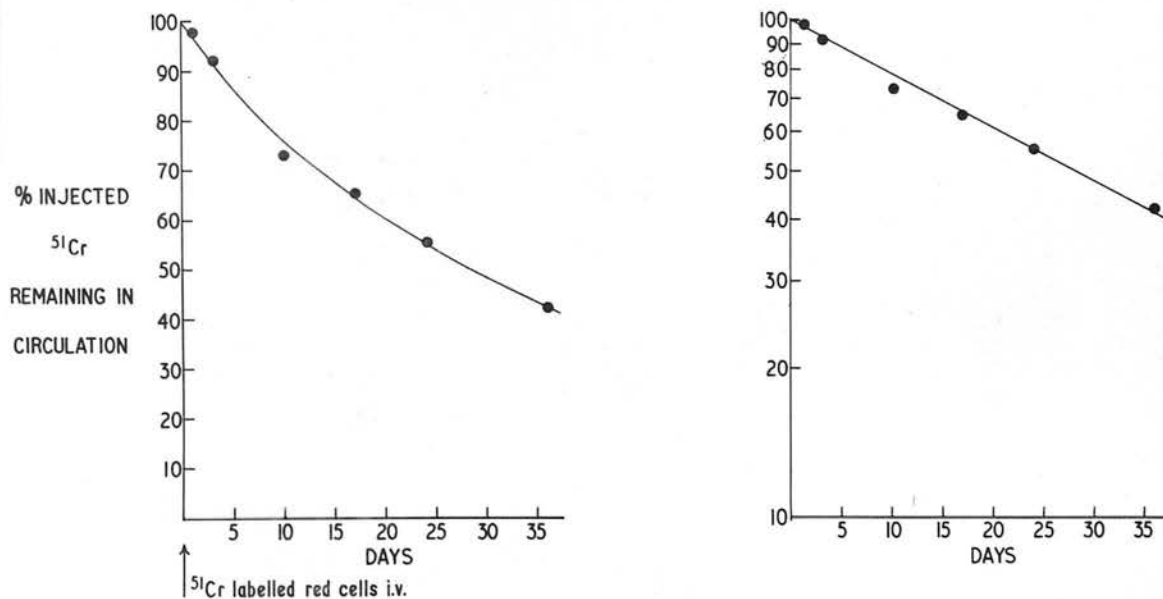


Fig. 5

Red cell survival data obtained by the chromium-51 method in one normal subject plotted on an arithmetical scale (left) and logarithmic scale (right).

function of time, is curvilinear. The results for each individual, when plotted on a semilogarithmic graph, approximated to a straight line and this is illustrated for one control subject in Fig. 5.

For these nine control subjects the linear correlation coefficient between the logarithm of the percentage of injected chromium-51 remaining in the circulation and time is -0.9887 ($p < 0.001$); the regression coefficient of the log. percentage ^{51}Cr remaining in the circulation on days is -0.0107 . The calculated average normal curve illustrated by the bold interrupted line in Fig. 4 is that of the equation:-

$$\text{Log. } y = 1.9851 - 0.0107x$$

This line will appear on subsequent illustrations for reference.

The $T_{1/2}\text{Cr}$ in these nine control subjects ranged from 24.5 to 31 days (mean 28 days).

The results given by Mollison and Veall (1955), whose method was being followed, were examined in the same way. It was found, using the data for the average percentage of ^{51}Cr remaining in the circulation in ten of their normal subjects for the first twenty days, that the regression coefficient was -0.01159 . The average percentage of injected ^{51}Cr at twenty-five days was 50.3.

It was realised at an early stage that results for blood/

blood radioactivity in patients with haemolytic disease when plotted on a semilogarithmic graph, seldom produced a straight line and hence comparison of data with that obtained in normal subjects could not be on the basis of regression coefficients. The results obtained using chromium-51 in various chronic diseases are illustrated in Fig. 6.

Reduction of red cell survival appears to vary from slight to moderate. The results obtained in various known haemolytic disorders are illustrated in Fig. 7. In view of the generally marked deviation of these curves from the normal, it was felt reasonable not to undertake elaborate analyses of the curves or to undertake correction for chromium elution but to use the $T_{1/2}^{Cr}$ as the basis for comparison.

Comparison of Ashby technique and chromium-51 technique in disease states.

Although, as has already been stated, the precise rate of elution of chromium is not important in the interpretation of measurements using chromium-51 labelled red cells when haemolysis is marked, it was felt that in order to have confidence in the use of the $T_{1/2}^{Cr}$ the assumption that the rate of elution was approximately the same in disease states as in health should be tested. Accordingly, simultaneous measurements were made of red cell survival using the Ashby technique and chromium-51 in three patients with symptomatic non-immune haemolytic anaemia. The chromium data were then corrected/

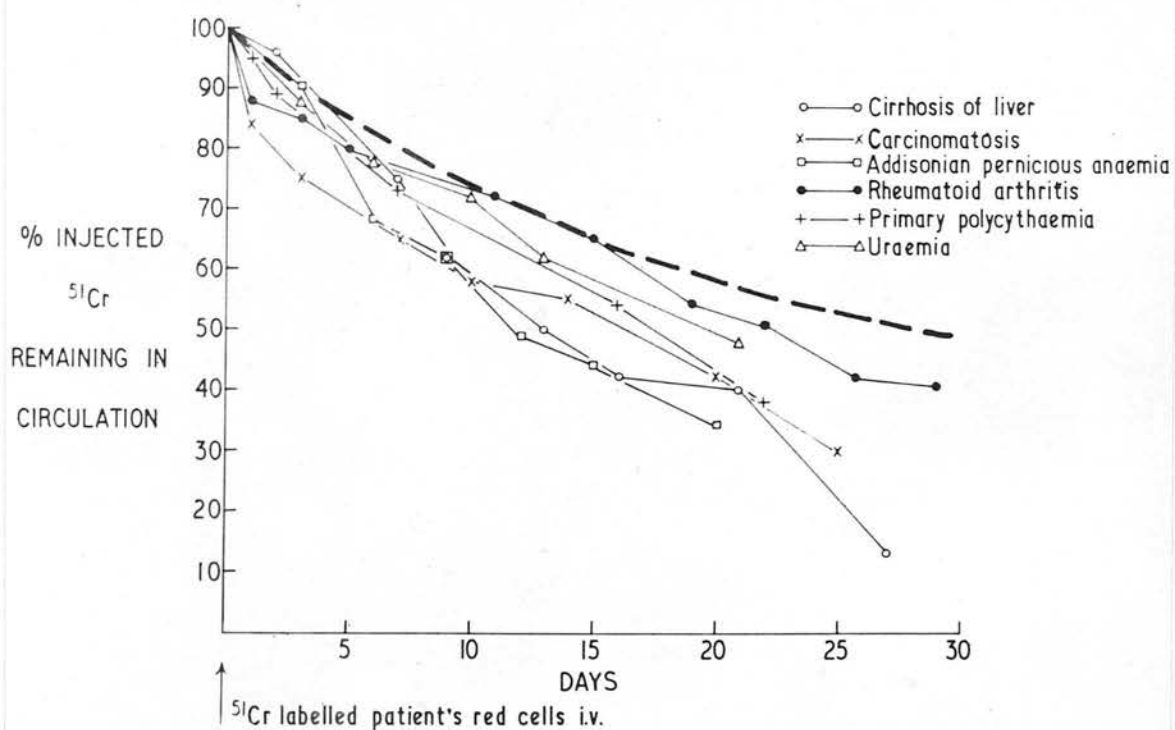


Fig. 6

Red cell survival measurements in various chronic and haematological diseases.

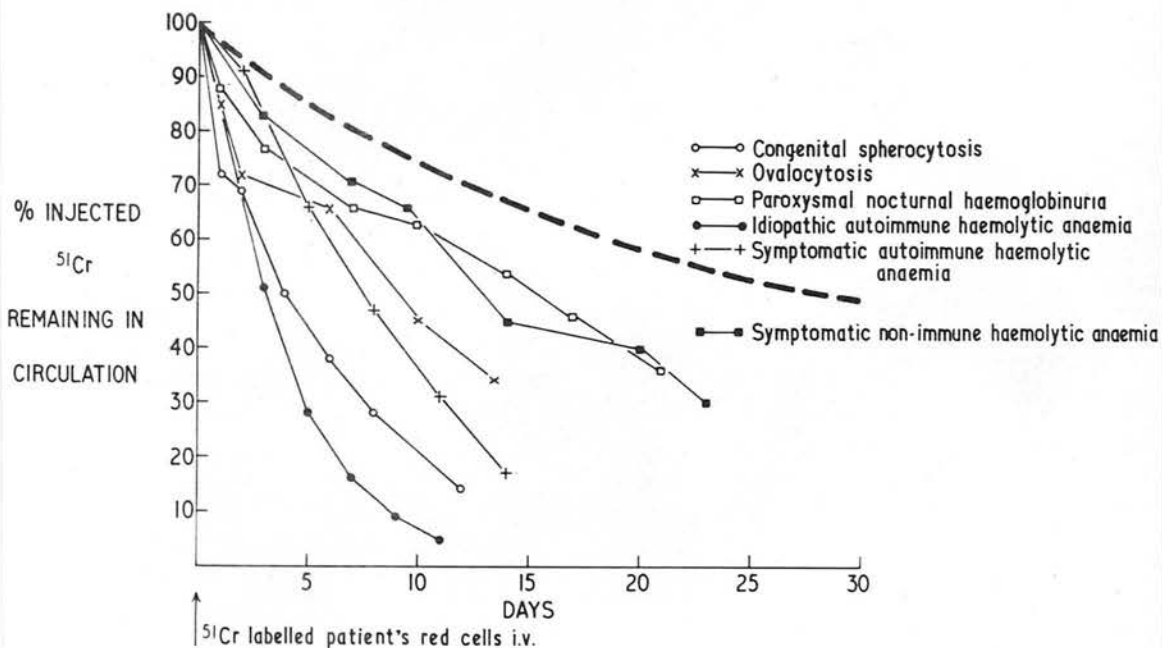


Fig. 7

Red cell survival measurements in various haemolytic disorders.

corrected, assuming that the elution of ^{51}Cr from the cells had a half period of seventy days. The results are illustrated in Fig. 8.

The close "fit" of the corrected chromium data with that obtained by the Ashby method was taken to support the validity of the use of the $\text{T}_{1/2}\text{Cr}$ for comparison of results that are discussed in this thesis.

Reproducibility of chromium-51 method.

Little information is available regarding the reproducibility of the chromium-51 survival measurements. Results in four subjects are illustrated in Fig. 9. The first graph shows the curve obtained in one normal individual (A), in whom the survival of his own red cells was compared with that of forty-eight hour old compatible donor red cells. Subject B, a patient with a mild haemolytic process due to rheumatoid arthritis, showed more rapid elimination of cells from a healthy donor than of his own red cells; this is a well known phenomenon (Mollison 1959) in acquired haemolytic disorders and is believed to be due to the wide distribution of red cells in terms of age and susceptibility to destruction in the donor compared with the patient; the latter's red cells probably comprise more young erythrocytes and cells that have resisted destruction. Subject C and Subject D both had symptomatic non-immune haemolytic anaemia; the red cell survival measurement was made on two occasions during/

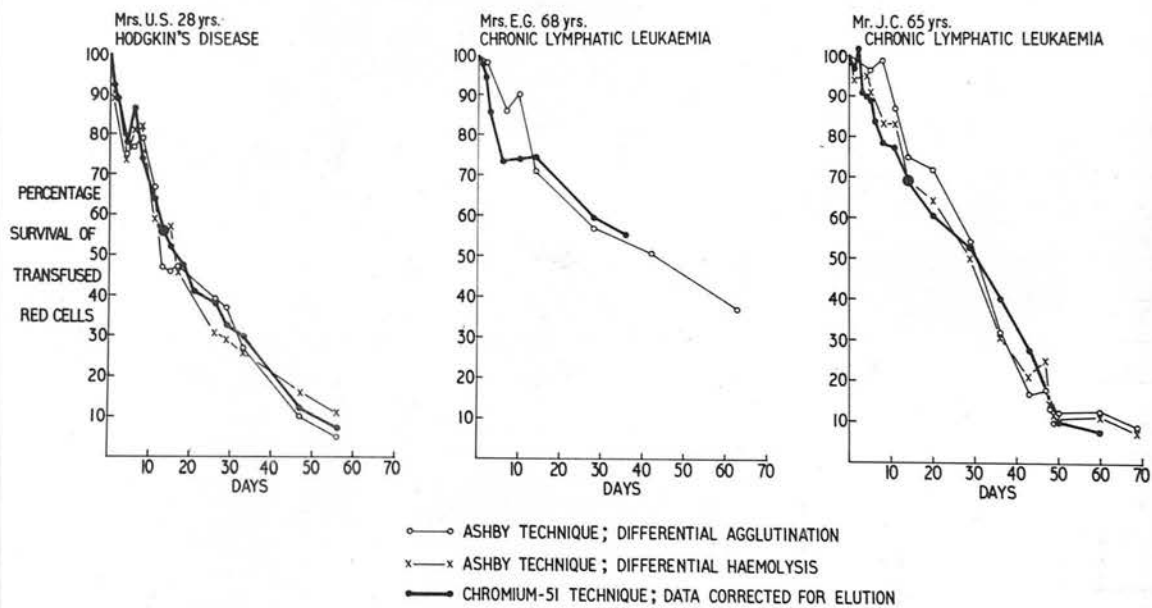


Fig. 8

Comparison of simultaneous red cell survival measurements by the Ashby technique and by the chromium-51 method in three patients with symptomatic non-immune haemolytic anaemia. The ^{51}Cr data have been corrected for chromium elution assuming an elution rate of half period 70 days.

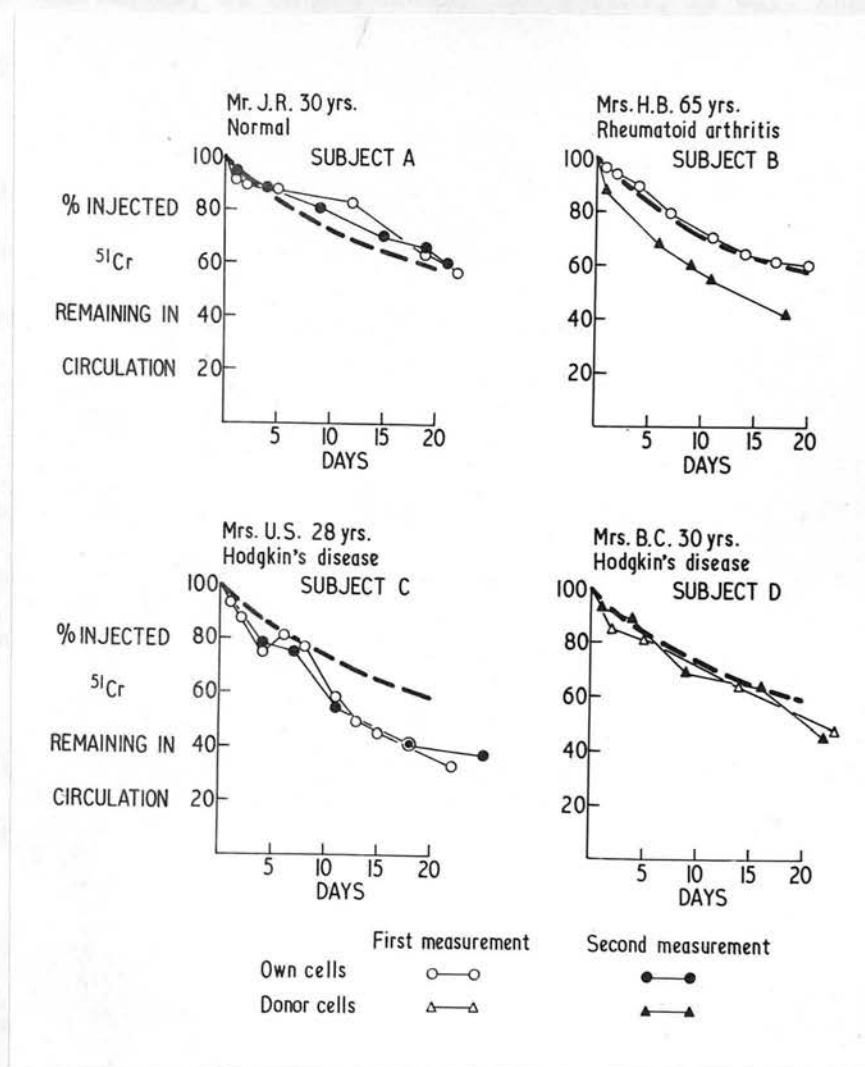


Fig. 9

Two measurements of red cell survival in four subjects. The reproducibility in terms of $T_{1/2}\text{Cr}$ in Subjects A, C and D is good. Subject B, a patient with rheumatoid arthritis, illustrates the more rapid destruction of erythrocytes from a healthy donor compared with his own as is usually seen in acquired haemolytic disease.

during a period when the haemoglobin level and haematocrit were stable. The high reproducibility of results in these circumstances, as in the normal individual, is well seen.

Interpretation of body surface measurements.

At an early stage in the collection of data on body surface radioactivity, an attempt was made to dissociate the radioactivity due to tissue uptake from that due to perfusing blood using the following formula:-

$$\text{Organ radioactivity on day } t = S_t - \left(S_o \times \frac{B_t}{B_o} \right)$$

where S = radioactivity over surface of organ, B = blood radioactivity, t = day t, and O is the measurement made one hour after injection of ^{51}Cr .

The effect of this procedure on the graphical presentation of data is illustrated in a typical case of symptomatic non-immune haemolytic anaemia in Fig. 10. This handling of the data did not contribute anything to the understanding of results and was not pursued. All the body surface measurements will be described using the spleen/praecordium and the liver/praecordium ratios, as originally suggested by Jandl, Greenberg, Yonemoto and Castle (1956), and the analysis of these data will be by the "spleen sequestration index" at $T_{\frac{1}{2}}\text{Cr}$ and the spleen/liver ratio at $T_{\frac{1}{2}}\text{Cr}$ (Fig. 10).

2. 7. Ancillary investigations.

Utilisation/

BLOOD, SPLEEN AND LIVER RADIOACTIVITY AFTER i.v. INJECTION OF ^{51}Cr LABELLED RED CELLS IN PATIENT WITH SYMPTOMATIC HAEMOLYTIC ANAEMIA

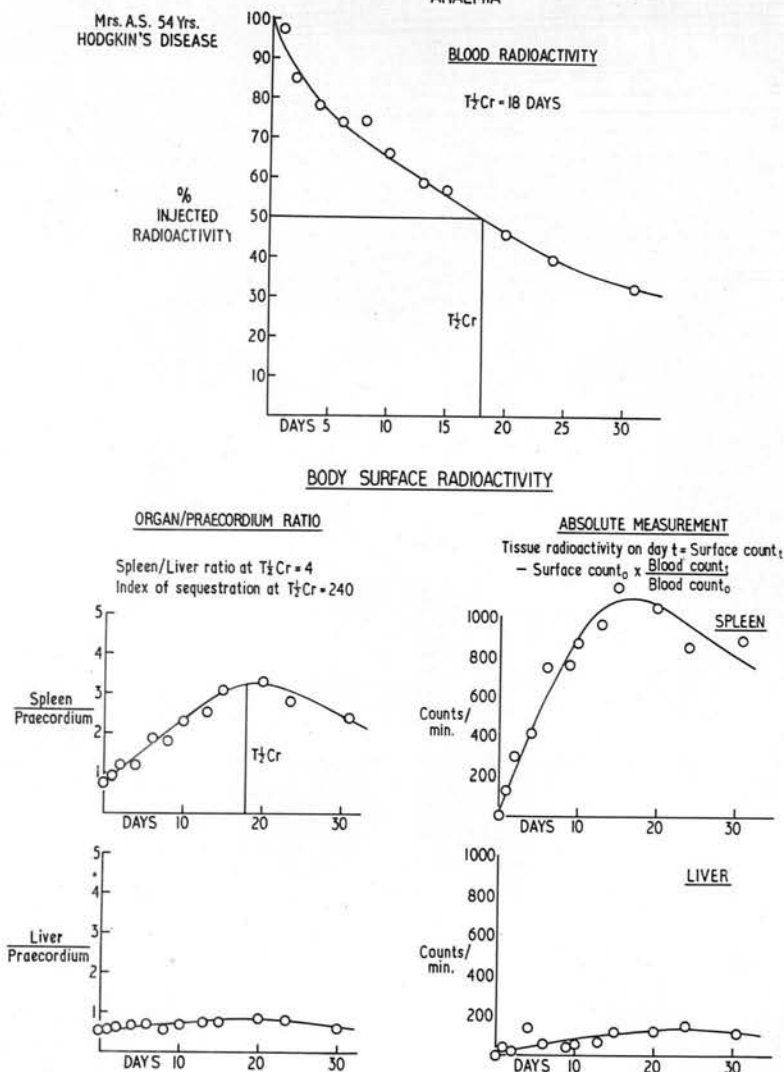


Fig. 10

Data obtained using chromium-51 in one patient regarding red cell survival and body surface measurements of radioactivity. The derivation of the spleen sequestration index and the spleen/liver ratio at $T_{1/2}\text{Cr}$ is illustrated. The absolute tissue radioactivity for liver and spleen have been estimated in this patient (see text) by subtracting that calculated to be due to perfusing blood; it is seen that these data do not add to the information that can be derived from the spleen/praecordium and liver/praecordium ratio.

Utilisation of haemopoietic factors.

Since the introduction of radioactive iron into routine haematological practice, a number of investigators have shown that, with the greatly increased marrow activity which accompanies haemolytic disease, there is a very rapid turnover of iron (Wetherley-Mein, Hutt, Langmead and Hill 1956, Giblett, Coleman, Pirzio-Biroli, Donohue, Motulsky and Finch 1956, Bothwell, Hurtado, Donohue and Finch 1957). Because of the body's ability to conserve and re-utilise iron, iron deficiency does not arise and haemoglobin synthesis is unimpaired. As in all anaemias not due to iron deficiency, there is evidence of accumulation of iron in the tissues; there is also in haemolytic disease, as in aplastic anaemia, elevation of the level of iron in the plasma. One measure of the change in ferrokinetics is to observe the rate of clearance of intravenously injected iron-59 labelled patient's plasma. This has been undertaken in six patients with haemolytic disease using the method of Veall and Vetter (1958) and the results are illustrated in Fig. 11. Whereas in normal subjects 50% of the injected radioactive iron is removed from the circulation in 70-120 minutes (Ledlie and Baxter 1954), in haemolytic disease the rate of elimination is greatly accelerated; in the six test subjects the half period of removal of iron ranged from 14.5 to 30 minutes (mean 20.4 minutes).

More/

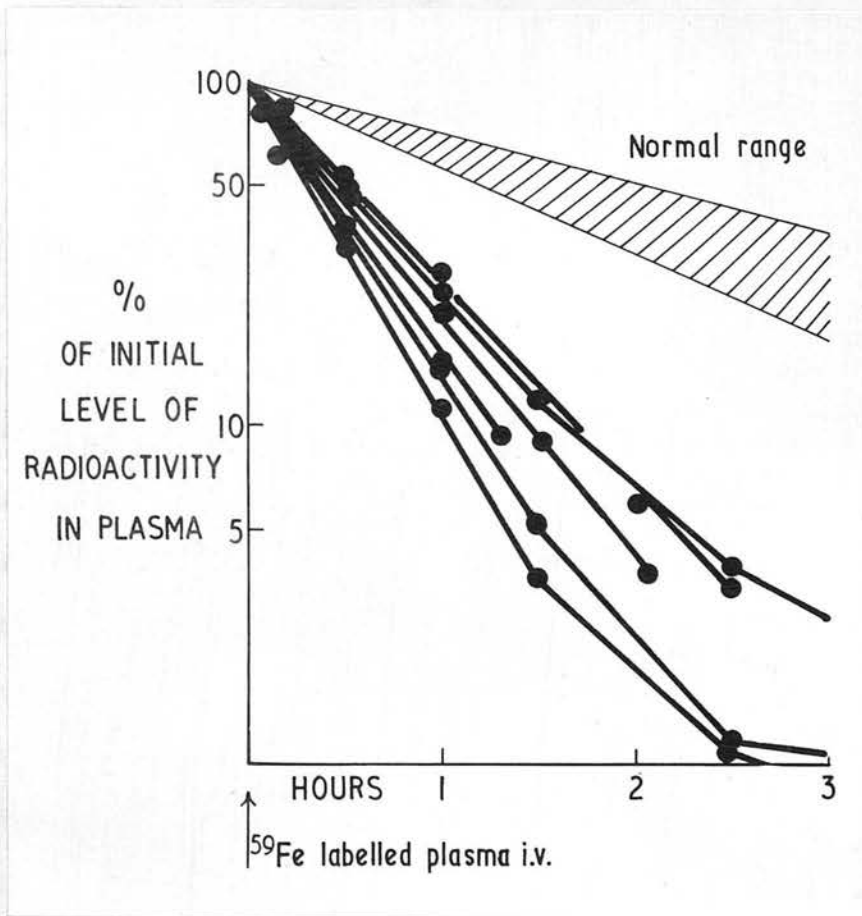


Fig. 11

The clearance of intravenously injected ^{59}Fe labelled plasma in 6 patients with haemolytic disease.

More interest attaches to the metabolism of folic acid and vitamin B₁₂. Clinical experience of these B vitamins does not suggest that they are readily conserved by the body or that they are re-utilised like iron, and although the body stores of vitamin B₁₂ may last for many months, it has been suggested that our supplies of folic acid may be depleted very rapidly (Vinke and Van der Sar 1956).

Reference has already been made to the occasional complication of haemolytic disease by megaloblastic erythropoiesis. The first account of this is believed to be that of Crosby and Sacks (1949) who described megaloblastic anaemia in a young man with thalassaemia. Additional examples of the association have been seen in acquired haemolytic anaemia (Baikie and Pirrie 1956), hereditary spherocytosis (Davidson 1952, Drury and Geoghegan 1957, Delamore, Richmond and Davies 1961), thalassaemia (Goldberg and Schwartz 1954, Jandl and Greenberg 1958), sickle cell anaemia (Jonsson 1958) and paroxysmal nocturnal haemoglobinuria (Heffernan and Jaswon 1955).

In a detailed study of three patients with megaloblastic erythropoiesis and a haemolytic disorder, Chanarin, Dacie and Mollin (1959) were able to show that the serum vitamin B₁₂ level was normal but in each case there was an abnormally rapid clearance of intravenously injected folic acid and the administration of folic acid caused conversion of the marrow picture to normoblastic erythropoiesis and correction/

correction of the reticulocytopenia which had developed.

In the present investigations the following measurements have been made:-

Serum Vitamin B₁₂ (L. Leichmannii method with added cyanide as described by Girdwood 1960).

17 patients;

Serum folic acid, by courtesy of Professor R. H. Girdwood and Dr. P. W. Kershaw (L. casei method as described by Waters and Mollin 1961).

12 patients;

Plasma clearance of injected folic acid, by courtesy of Professor R. H. Girdwood (Method of Chanarin, Mollin and Anderson 1958).

9 patients.

The results of these tests are illustrated in Figs. 12+13

It will be seen that the rate of clearance of injected folic acid from the plasma is increased in all but one patient. However, in only three patients (all of whom showed megaloblastic erythropoiesis) is the serum folic acid level outwith the normal range, and likewise in only three patients (one of whom showed megaloblastic erythropoiesis) is the serum vitamin B₁₂ level subnormal. The implications of these changes in vitamin B₁₂ and folic acid metabolism will be discussed in relation to the patients marked with an asterisk on pages 69 and 181.

Plasma cholesterol

The chance finding of low levels of cholesterol in the plasma in patients with congenital spherocytosis led to a study/

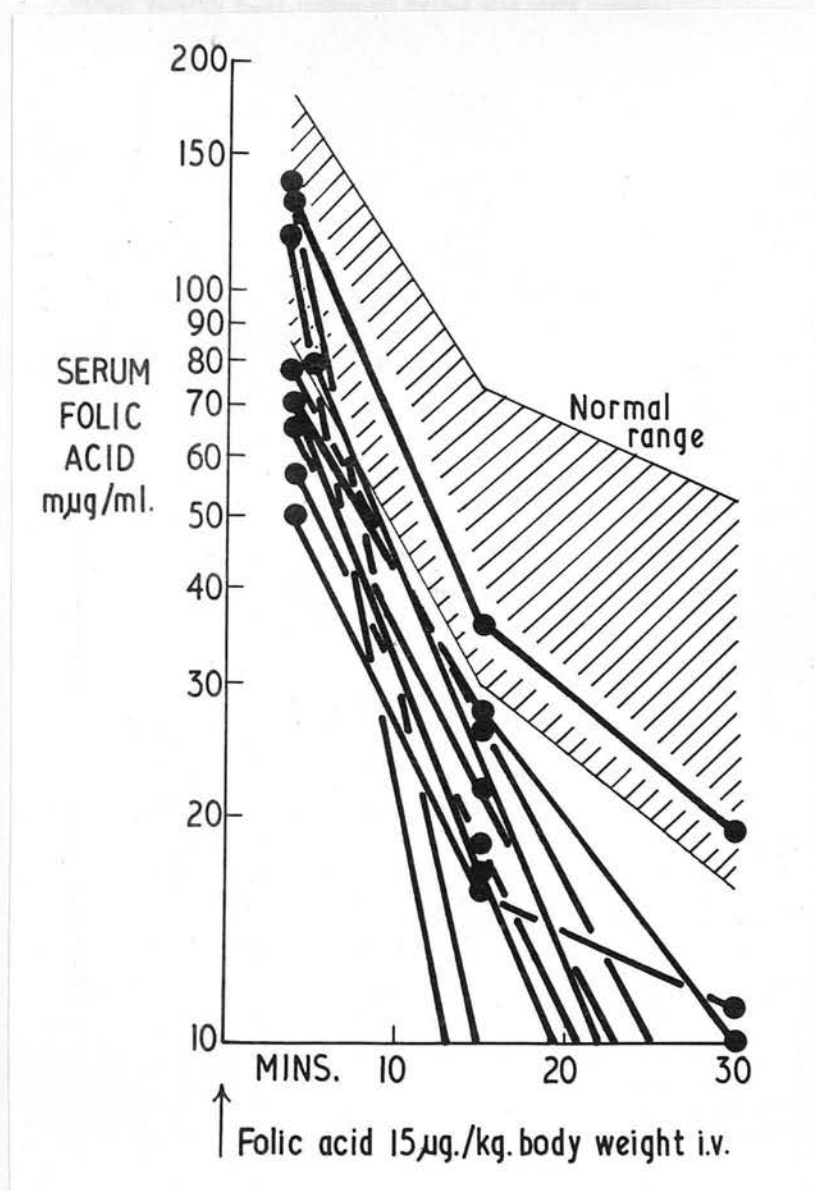
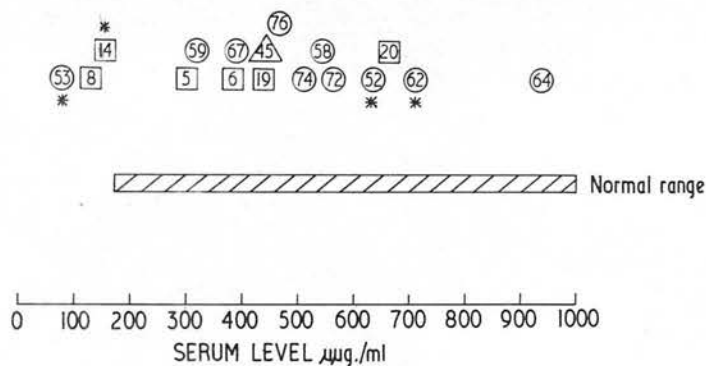


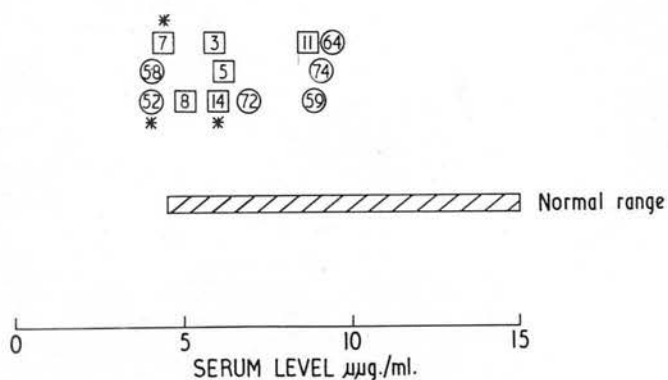
Fig. 12

The clearance of intravenously injected folic acid
in 9 patients with haemolytic disease.

SERUM VITAMIN B₁₂ (L.leichmannii method with added cyanide)



SERUM FOLIC ACID (L.casei method)



□ Autoimmune haemolytic anaemia

△ Symptomatic non-immune haemolytic anaemia

○ Congenital spherocytosis

* Patients showing megaloblastic erythropoiesis

Fig. 13

The serum Vitamin B₁₂ and folic acid levels in patients with haemolytic disease. The enclosed figures are Case Nos.

study of the incidence of this abnormality and of the effect of arresting the haemolytic process by splenectomy. The results are discussed on page 203. The estimations were undertaken in the Biochemistry Department, Royal Infirmary of Edinburgh, by courtesy of Dr. C. P. Stewart and Dr. J. A. Owen, using the methods of Sackett (1925) and Searcy and Berquist (1960).

CHAPTER THREE

AUTOIMMUNE ACQUIRED HAEMOLYTIC ANAEMIA3. 1. Introduction

There is an extensive literature referring to the incidence of overt haemolytic anaemia with reticulocytosis and acholuric jaundice, complicating various haemopoietic disorders, diseases of connective tissue and miscellaneous conditions such as infections and ovarian tumours. There is particular emphasis on this occurrence in malignant lymphocytic disease (Rosenthal, Pisciotto, Komninos, Goldenberg and Dameshek 1955) and Hodgkin's disease (Wasserman, Stats, Schwartz and Fudenberg 1955), and attention has been drawn to the fact that it may be the first evidence of disseminated lupus erythematosus (Sarles and Levin 1959). In these diseases a clinically significant symptomatic haemolytic anaemia may be much more common than is generally realised; Wasserman and his colleagues (1955) estimate, for example, that it dominates the clinical picture in 15 per cent of patients with chronic lymphatic leukaemia. Many haematologists are also aware of the frequency of haemolytic disease in myeloid metaplasia and the problems of management which this creates (Cartwright, Finch, Loeb, Moore, Singer and Dameshek 1955). It seems on the other hand that while severe haemolysis may present in various other reticuloses and leukaemias, in other connective tissue/

tissue diseases, in virus pneumonia and infectious mononucleosis and in dermoid cysts and ovarian tumours, this is much less common than in the conditions referred to. The aetiological relationship of the underlying disorder can not always be proved but is strongly suggested by remission of all signs of excessive blood destruction following successful treatment of the primary disease, e.g. after the giving of nitrogen mustard in Hodgkin's disease (Sievers and Harwerth 1953) or after the surgical removal of an ovarian tumour (Allibone and Collins 1951).

Many of the studies of symptomatic haemolytic anaemia were undertaken before serological studies were widely applied. The more recent reports indicate that the direct antiglobulin test is frequently positive (Müller and Schuboth 1958) and the basis for the haemolytic process is an "autoimmune" reaction, but experience of large numbers of patients (Dacie 1959, 1962) indicates that a positive Direct Coombs' test is only common in diseases of lymphoid tissue, virus infections and lupus erythematosus, being rare in the other conditions of the symptomatic group. In the present state of knowledge, this type of haemolysis in which red cell antibodies cannot be demonstrated has to be regarded as "non-immune" and it is the basis for the considerations in the next chapter.

Despite these several diseases which are associated with the development of auto-erythrocyte antibodies, there is/

is still a majority of patients with autoimmune haemolytic anaemia in whom there is no demonstrable underlying disease; it is to them that the unsatisfactory term "idiopathic" is applied. Idiopathic autoimmune haemolytic anaemia may be a cumbersome term but it is probably retained for the want of something more descriptive and also because it emphasises the fact that the reason why some individuals spontaneously generate antibodies against constituents of their own red corpuscles is still unknown.

Information is accumulating on the relative incidence of symptomatic autoimmune haemolytic anaemia as compared with the idiopathic variety. Lal and Speiser (1957) found 48 of their 97 cases of the warm antibody type to be symptomatic; van Loghem, van der Hart and Dorfmeier (1958), 67 of 122 cases; Revol, Lejeune, Brizard, Jouvenceaux and Perrin (1958) 7 of 27 cases; and Dausset and Colombani (1959), 35 of 128 cases. In Dacie's (1962) series of 175 cases, 108 were found to be of the idiopathic variety and 59 symptomatic; the remaining 8 patients, who had paroxysmal cold haemoglobinuria (sometimes referred to as the "cold haemagglutinin syndrome"), were separated because of their different clinical behaviour. The relative incidence of the warm and cold antibody type of autoimmune haemolytic anaemia has also been studied in large series; Dausset and Colombani (1959) found the ratio of warm to cold type in the idiopathic group was 8:1 and in the symptomatic group 2:1; Dacie's/

Dacie's (1962) results (excluding the patients with paroxysmal cold haemoglobinuria) show a ratio of warm to cold type in the idiopathic group of 4.7:1 and in the symptomatic group of 2:1. The need to distinguish between these two types of antibody is based on clinical experience; while a cold antibody may cause a haemolytic process identical with that of the warm type, it is most frequently seen in high titre in the serum in elderly subjects when increased blood destruction is only a minor part of the clinical picture (Dacie 1962).

Autoimmune haemolytic anaemia has been most frequently described in Caucasian subjects and is not generally regarded as having any familial basis. However, Dacie (1962) refers to an account by Kissmeyer-Nielson, Bent-Hansen and Kieler (1952) of the development of an autoimmune type of haemolytic anaemia in mother and daughter. The idiopathic disease seems to affect all ages and in an analysis of 125 of his own cases of autoimmune haemolytic anaemia of warm antibody type by Dacie (1962), the youngest patient was five months and oldest 78 years at the time of first diagnosis. Most authors have found an excess of female patients in their series. For example, Lal and Speiser (1957) found that 55% of 49 patients with idiopathic disease and 62.5% of 48 patients with symptomatic disease were females. In Dacie's (1962) series, the females accounted for/

for 58% of 108 cases of idiopathic disease and 66% of 59 patients in the symptomatic group.

3. 2. Composition of the group of patients with autoimmune haemolytic anaemia and mode of presentation.















Twenty-three patients with autoimmune haemolytic anaemia have been studied; the main details at the time of diagnosis are summarised in Table 5. Of this group, the disease was of the idiopathic variety in 16 and symptomatic in 7. The primary condition in the latter patients was lympho-proliferative disease in 4, disseminated lupus erythematosus in 2, and an unusual reticulosis, which has been described in the literature (Farquhar and Claireaux 1952 and Farquhar, MacGregor and Richmond 1958) and termed "familial haemophagocytic reticulosis", in one.

In both the idiopathic group and the symptomatic group, there was a wide age distribution, being 9 months to 76 years in the former and 4 months to 64 years in the latter. Twelve of the 16 patients (75%) with idiopathic disease were females, whereas only 2 of the 7 (29%) with symptomatic disease were female patients.

The duration of symptoms prior to diagnosis was extremely variable. Six patients (one in the symptomatic group) presented with a haemolytic crisis; in two children (Cases 4 and 21), non specific symptoms, notably anorexia, had/

TABLE 5

CLINICAL DETAILS OF PATIENTS WITH AUTOIMMUNE HAEMOLYTIC ANAEMIA AT TIME OF DIAGNOSIS

Patient	Age	Sex	Primary Disease Causing Haemolytic Process	Duration of Symptoms Before Diagnosis	Mode of Presentation	Physical Findings at time of Diagnosis		
						Pallor	Icterus	Splenomegaly
1. M.B.	73	F	--	Many years	Icterus Raynaud's phenomena	0	+	0
2. H.D.	61	M	--	8 months	Slowly progressive anaemia	+	+	
3. M.F.	35	F	--	3 days	Acutely developing anaemia	++	+	Splenectomy 1956
4. J.F.	9 months	F	--	2 months	Anorexia Discoloured urine	+	+	0
5. C.H.	46	F	--	7 months	Jaundice	+	+	
6. I.H.	72	F	--	6 months	Progressive anaemia	+	+	
7. H.L.	24	F	--	18 days	Acutely developing anaemia	++	+	0
8. M.L.	71	F	--	18 months	Incidental finding of splenomegaly during respiratory infection	+	+	
9. N.L.	31	F	--	2 weeks	Acutely developing anaemia	+	+	
10. E.L.	48	F	--	3 months	Progressive anaemia	+	+	
11. I.L.	76	F	--	3 years	Anaemia and jaundice	+	+	
12. M.M.	48	F	--	3 weeks	Acutely developing anaemia	+	+	
13. H.O.	65	M	--	Many years	Fatigue Raynaud's phenomena	+	+	
14. J.R.	50	M	--	3 months	Progressive anaemia	+	+	0
15. C.S.	63	M	--	14 months	Fatigue following pneumonia	0	+	0
16. I.S.	27	F	--	2 days	Acutely developing anaemia	+	+	0
17. A.B.	52	M	Lymphosarcoma	8 months	Progressive anaemia Pruritus	+	0	
18. I.E.	32	F	Lupus erythematosus	12 months	Recurrent anaemia Pleural effusion	0	0	0
19. G.D.	62	M	Lymphosarcoma	2½ years	Acutely developing anaemia	+	+	First seen in remission
20. D.M.	64	M	Giant follicle lymphoma	3 years	Cold haemoglobinuria	+	0	
21. I.M.	4 months	M	Familial haemophagocytic reticulosis	4 weeks	Progressive anaemia	+	0	
22. J.S.	49	M	Lymphosarcoma	12 months	Anorexia and irritability	+	0	
23. E.W.	30	F	Lupus erythematosus	2 years	Progressive anaemia Arthralgia	+	+	

had been present for one month and two months respectively; two elderly patients (Cases 1 and 13) who suffered from the "cold haemagglutinin syndrome" had complained of Raynaud's phenomena for many years; in the remainder the symptoms of anaemia had been developing insidiously for periods up to three years. Only in one patient (Case 23) in the symptomatic group did the diagnosis of the primary disease antedate the recognition of the haemolytic process. In Case 8 it was the accidental finding of splenomegaly that led to diagnosis.

Clinical anaemia was found in all but three patients at the time of first examination; one of these three was in partial remission when the diagnosis was made. Clinical icterus was encountered slightly less frequently, being definitely present in 15 patients, doubtful in 3 patients and absent in the remaining 5.

The physical findings relating to the spleen during the initial illness are detailed and illustrated in Table 5. The only patient showing gross splenomegaly was Case 17, a man with lymphosarcoma in whom the great enlargement of the spleen would almost certainly be due to infiltration with the primary disease. In the idiopathic group, moderately enlarged spleens were found in only three patients, being approximately 10-15 cm. below the left costal margin. In 7 of the 16 patients in this group there was, however, no palpable enlargement of the spleen at the time of diagnosis; one/

one of these was a patient (Case 1) with the cold haemagglutinin syndrome, and splenomegaly is known to occur in only a minority of these subjects (Dacie 1962); four patients without splenomegaly had a history of less than three months but in the other two the haemolytic process appeared to have been present for three years (Case 11) and fourteen months (Case 15) respectively.

With the exception of Case 21, there was no evidence of any familial predisposition to haemolytic disease. Other haematological disorders featured in the family history of only three patients; the mother of Case 4 suffered from hereditary haemorrhagic telangiectasia, the mother of Case 9 had proven Addisonian pernicious anaemia and the father and sister of Case 10 also have definite pernicious anaemia.

3. 3. The main haematological and biochemical findings at the time of diagnosis.

The data discussed in this section are summarised in Table 6.

Peripheral blood examination.

As would be expected, the haemoglobin level at the time of diagnosis varied within a wide range from 21% to 85% in the idiopathic group and 30% to 84% in the patients with symptomatic haemolytic disease. As is the general experience, the two patients with the "cold haemagglutinin syndrome" (Cases 1 and 13) had relatively mild haemolytic disease/

TABLE 6

HAEMATOLOGICAL AND BIOCHEMICAL FINDINGS IN PATIENTS WITH AUTOIMMUNE HAEMOLYTIC

Patient	Hb% Retics.%	E.S.R. mm/hr.	Other Features in Peripheral Blood	Serum Bilirubin mg/100 ml.	Urobilinogenuria *	Liver Function Tests **	Plasma Proteins g/100 ml
1. M.B.	80	7	Haemagglutination at room temperature	4.5	Trace	Normal	A 4.5 G 1.9
2. H.D.	47	30	Marked spherocytosis and increase in osmotic fragility Normoblasts	2.4	+++	Normal	---
3. M.F.	34	2.6	Platelets < 10,000 Thrombocytopenia Macrocytosis Microscopic haemagglutination W.B.C. 44,000	2.6	++	Normal	A 3.2 α ₁ O. β 0.6 γ 1.1
4. J.F.	52	30.5	Spherocytes and normoblasts	1.4	0	---	---
5. C.H.	48	36.5	Spherocytes and normoblasts	2.6	++	Normal	A 3.6 α ₁ O. β 0.5 γ 0.8
6. I.H.	51	20	Marked anisopoikilocytosis Normoblasts	2.6	+	Normal	A 4.5 α ₁ O. β 0.8 γ 0.9
7. H.L.	21	15	Microscopic haemagglutination Normoblasts Myelocytes Spherocytes and increased osmotic fragility W.B.C. 24,700	2.1	+	Normal	A 2.8 α ₁ O. β 0.4 γ 0.8
8. M.L.	50	32.6	Spherocytes (Possible megaloblastic erythropoiesis in bone marrow)	2.4	+++	Alkaline phosphatase 18 K.A. units	A 4.2 α ₁ O. β 0.5 γ 1.1
9. N.L.	30	25	Platelets 145,000/c.mm.	1.1	+++	---	---
10. E.L.	44	15.5	Spherocytes	3.5	+	Normal	A 3.9 α ₁ O. β 0.6 γ 1.1
11. I.L.	43	21.7	Spherocytes	2.8	+++	Normal	A 2.9 α ₁ O. β 1.3 γ 1.7
12. M.M.	42	27.0	Microscopic haemagglutination Spherocytes Normoblasts Myelocytes	3.0	++	Normal	A 2.8 α ₁ O. β 0.6 γ 2.2
13. H.O.	85	2.0	Haemagglutination at room temperature Lymphocytosis (65% of 19,000 white cells) Platelets 115,000	2.1	0	Normal	A 3.8 α ₁ O. β 0.9 γ 0.8
14. J.R.	50	19.6	Microscopic haemagglutination Spherocytes Normoblasts (Possible megaloblastic erythropoiesis in bone marrow)	1.0	++	Normal	A 4.3 α ₁ O. β 0.6 γ 0.8
15. C.S.	55	3.1	Spherocytes	0.6	0	Normal	A 3.0 G 2.3
16. I.S.	34	4.0	Spherocytes W.B.C. 42,100	6.0 --> 2.5 on second day	0 --> +++ on second day	Normal	A 4.9 G 3.1
17. A.B.	43	3.3	Lymphocytosis (61% of 4,400 white cells) Platelets 85,000 (Lymphocytic infiltration in bone marrow) LE cells present	0.4	0	Normal	A 3.7 α ₁ O. β 0.6 γ 1.2 (macroglobulin α ₂ zone)
18. I.E.	84	3.3	LE cells present	1.0	++	Zinc sulphate turbidity 12 units rhymol flocculation positive	A2.8 α ₁ O.4 β 0.7 γ 2.8
19. G.D.	49	13.0	Spherocytes Normoblasts (Possible megaloblastic erythropoiesis and lymphocytic infiltration in the bone marrow)	1.7	++	Normal	A 3.8 α ₁ O. β 0.4 γ 0.2
20. D.M.	65	4.1	---	1.5	0	S.G.P.T. 67 units	A 2.9 α ₁ O. β 0.8 γ 0.5
21. I.M.	65	6	16% unrecognisable cells in peripheral blood Thrombocytopenia; 32,000 Erythrophagocytosis in bone marrow	---	++	---	---
22. J.S.	74	< 1	Lymphocytosis; 81% of 3,000 cells. Platelets 15,000 Hypoplastic bone marrow	0.7	Trace	Normal	A 3.8 G 2.3
23. E.W.	30	9	LE cells present	1.6	+	Normal	A 3.0 α ₁ O. β 0.6 γ 2.1

* The urinary content of urobilinogen has been graded as follows:- 0, trace, +, ++, +++.

** Liver function tests have included measurement of the serum alkaline phosphatase level and reactions in addition to estimates of the serum bilirubin level and protein electrophoresis. 12, 15, 17, 19 and 20, the S.G.O.T. and S.G.P.T. levels were also measured.

*** A = albumin; G = globulin; α₁, α₂, β, γ refer to the principal globulin fractions estimated

disease and presented with haemoglobin levels of 80% and 85% respectively. Excluding these two patients, Case 18 who was in partial remission when first seen and Case 22 in whom the diagnosis depended on red cell survival measurement (Table 6), significant anaemia was always present, the lowest haemoglobin levels being found in the six patients whose illness began with a haemolytic crisis (Case 3, 34%; Case 7, 21%; Case 9, 30%; Case 12, 42%; Case 16, 34%; Case 19, 49%). With the exception of Case 22 (who had a hypoplastic bone marrow) and Case 13 (cold haemagglutinin syndrome), a reticulocyte count in excess of 2% was present in all the patients with autoimmune haemolytic anaemia; the figures ranged from 2.6% to 36.5%, and this reticulocytosis formed a most useful diagnostic indicator in the early stages of illness. A reticulocytosis of 68% was recorded in Case 5 during the first few days of investigation. No correlation existed in the group of patients between the duration of the history (Table 5) or the severity of the haemolytic process as measured by the $T_{1/2}Cr$ and the reticulocytosis; the most acute illness was seen in Cases 3 and 16, and their reticulocyte counts were initially only 2.6% and 4% respectively, but this may merely reflect the delay of the marrow in beginning compensatory activity in a previously healthy subject.

It/

It is evident (from Table 6) that spherocytosis and the appearance of primitive red and white cells in the circulation is extremely common. Spontaneous haemagglutination was also noted relatively frequently, occurring in the blood collecting tube in the two patients with the cold haemagglutinin syndrome, and being found in blood films or during attempts to perform a red cell count in Cases 3, 7, 12 and 14. One peripheral blood finding is, however, at variance with other experience. Dacie (1962) states, from his extensive studies of autoimmune haemolytic disease, that "in acute haemolytic episodes, however caused, leucocytosis is characteristic, unless there is concomitant marrow failure". However, in only 4 of the 16 patients with idiopathic disease was the leucocyte count raised in the examinations of peripheral blood that led to diagnosis. One of these patients was Case 13 (cold haemagglutinin syndrome), and in another (Case 3) the leucocyte counts was probably influenced by previous splenectomy. The two other patients with high white cell counts (Cases 7 and 16) were among the group who had had a haemolytic crisis; Cases 9, 12 and 19 had also had a haemolytic crisis but their initial leucocyte counts were 6,600, 8,800 and 5,000 respectively.

The erythrocyte sedimentation rate (E.S.R.) is worthy of comment. This is frequently high in patients with autoimmune haemolytic anaemia and the tendency to spontaneous haemagglutination/

haemagglutination seems to be one possible explanation for this occurrence. It will be seen from Table 6 that in only three patients with idiopathic disease was the value less than 45 mm. in the first hour. In seven patients it was 100 mm. or more and the mean figure for the idiopathic group was 82 mm. This is in contrast to the finding in congenital spherocytosis (p. 175), in which condition the value tends to be low. Since acholuric jaundice, splenomegaly, reticulocytosis and spherocytosis are common to both the congenital disease and autoimmune haemolytic anaemia, this disparity in the E.S.R. level is also a useful indicator in the early steps towards diagnosis.

Thrombocytopenia was observed in three patients of the idiopathic group and three patients in the symptomatic group. The severe thrombocytopenia in Case 3 presented a unique problem and will be the subject of special discussion on p. 113.

The bone marrow

Smears of bone marrow aspirate (bone marrow trephine was necessary in Case 17) were examined in all patients. In the symptomatic group this was of diagnostic value in three of the patients, showing lymphocytic infiltration in Cases 17 and 19 and lymphocytic infiltration and hypoplasia in Case 22.

Megaloblastic erythropoiesis was demonstrated in Case 8, Case/

Case 14 (a patient who had previously had a gastroenterostomy) and Case 19. Case 7 (who is the subject of special discussion on p. 108) developed megaloblastic erythropoiesis at a later stage of illness at the time of a haemolytic crisis in the early stages of pregnancy. The details of vitamin B₁₂ and folic acid metabolism, in the period of the marrow finding, that are available for these patients are given in Figs. 12 and 13. In Cases 8 and 14 the serum vitamin B₁₂ level was subnormal, being 130 and 133 µg./ml. respectively; in Case 19 the serum vitamin B₁₂ level was 433 µg./ml. and conditioned deficiency of folic acid is presumed. In Case 7 folic acid deficiency is likely to have been the main factor since the serum folic acid level was depressed to 4.45 µg./ml. (normal ranged 4.5 to 15.0 µg./ml.).

The red cell survival measurements.

Red cell survival measurements were undertaken in 18 of the 23 patients. The T_{1/2}Cr ranged from 1.5 to 17 days (mean 11.7 days). Individual results are listed in Table 6. Further consideration is given to the data obtained with chromium-51 on p. 101.

Bile pigment metabolism and liver function.

The average serum bilirubin level at the time of diagnosis in the 22 patients with autoimmune haemolytic anaemia in whom the measurement was made was 2.16 mg./100 ml. The/

The level ranged from 0.6 to 6.0 mg./100 ml. but in only two patients (Cases 1 and 16) did it exceed 3.5 mg. This is in accord with the general experience that serum icterus is not severe in haemolytic disease. Allusion has already been made to the implication of liver function and red cell mass in the levels of bilirubin in the serum and the amount of urobilinogen in the urine, but it remains difficult to explain why in Cases 14, 15, 17, 18 and 22 the serum bilirubin should be normal and why in Cases 4, 13, 15, 17 and 20 there should be no increase of urobilinogen in the patient's urine. The only theories that can be advanced in explanation of these findings is that the excretory capacity of the liver for bile pigment is equal to the demand in these particular patients, or that in these patients haemoglobin is catabolised beyond the bilirubin and urobilinogen stage to compounds such as dipyrroles that are not being measured by present techniques. The work of Bingold (1949) indicates the latter possibility.

With the exception of Cases 4 and 9, in whom no data were available, and Case 8, who had a slight unexplained elevation of the serum alkaline phosphatase, the conventional tests of liver function gave normal results in all patients with idiopathic disease. In the symptomatic group changes in hepatic function are difficult to interpret since it is difficult to exclude effects attributable to the primary/

primary disease; however, only two of the patients in this group showed abnormalities and these are detailed in Table 6.

Plasma proteins.

No plasma protein pattern has been found to be typical of autoimmune haemolytic disease. Various minor abnormalities have been reported in small series of patients by Charbonnier and Dausset (1953), Fine, Vincon, Eyquem and Groulade (1954), Hennemann and Gillert (1954) and Sussman and Sang (1957). The largest series has been described by Christenson and Dacie (1957); of 38 patients suffering from idiopathic disease most showed normal plasma proteins but a tendency was found for the albumen and α_2 and β globulin fractions to be low; of 9 patients with symptomatic haemolytic anaemia one showed macroglobulinaemia and two with lupus erythematosus had hypergammaglobulinaemia.

The result of serum protein electrophoresis is available in 15 of the 23 patients with auto-immune haemolytic anaemia; 10 of these were in the idiopathic group and in none of these patients was any abnormality found. Changes that could be ascribed to the primary disease were found in the symptomatic group; both patients with disseminated lupus erythematosus (Cases 18 and 23) showed hypergammaglobulinaemia, Case 17 (with lymphosarcoma) had secondary macroglobulinaemia and Case 19 (with lymphosarcoma) had secondary hypo-gammaglobulinaemia.

Blood/

Blood groups

Several reports have appeared of the incidence of the different ABO blood groups in relation to autoimmune haemolytic anaemia. Hunt and Lucia (1953) found that 78% of 27 patients with acquired haemolytic disease were group O compared with 45% of their controls; Clemens and Walsh (1954-55) also obtained a significantly higher incidence of group O, 62% of their 66 patients with positive direct Coombs' tests being so classified. Information from larger series has however tended to refute these early observations. In Lal and Speiser's (1957) series group O accounted for only 39% of 97 patients and Dunsford and Owen (1960) reported group O in 47.2% of their 127 patients. Dacie (1962) gives the following incidence of blood groups for 120 patients: group O, 40%; group A, 44%; group B, 13.5%; and group AB, 2.5%. The results for the present series also suggest that the incidence of any particular ABO blood group is not significantly increased in patients with autoimmune haemolytic anaemia: group O, 12 patients, 52%; group A, 9 patients, 39%; group B, 1 patient, 4.5%; group AB, 1 patient, 4.5%.

Nature of the red cell antibody

Reference has already been made to the two patients with the cold haemagglutinin syndrome. In one patient with symptomatic/

symptomatic disease a positive direct antiglobulin test was not obtained in the period of investigation prior to death; it has, however, been felt justified to include him in this chapter on autoimmune haemolytic anaemia since he clearly suffered from a familial reticulosis (Case 4 of Farquhar, MacGregor and Richmond 1958), which had been responsible for the production of auto-antibodies in siblings. This family is discussed on p. 101. In the remaining 20 patients the red cell antibody was of warm type in 12 and of cold type in 8; in the latter number 2 patients appeared to have multiple auto-antibodies of which a cold type incomplete agglutinin appeared to predominate.

3. 4. The methods of treatment of autoimmune haemolytic anaemia.

Blood transfusion.

It is now believed that the therapeutic effect of transfusion, reported by Lederer (1925, 1930), is not a frequent occurrence. Although he considered that 11 of his 12 patient had responded, it seems likely from present experience that transfusion merely corrected the acute anaemia of the initial illness and that spontaneous remission was the major factor. One early problem in the management of autoimmune haemolytic anaemia does, however, relate to blood transfusion. By the nature of the haemolytic process, transfused blood cells are likely to suffer the same fate as the patient's own, and evidence points to a more/

more rapid rate of destruction of the donor cells than of those already circulating (Mollison 1959); this increases the amount of haemoglobin breakdown products that have to be metabolised and excreted. An exception exists where the auto-antibody is specific for a particular blood group substance, e.g. anti-c, anti-e; the transfusion of blood which does not contain the antigen in question would then be expected to have a normal survival in patients with this finding. (In the examples cited, blood of Rh genotype CDE/CDE if available should be suitable.) Transfusion studies in one patient with a specific auto-antibody (Ley, Mayer and Harris 1958) confirm that normal survival of blood of restricted genotype can be obtained. However, the propensity which patients with autoimmune haemolytic anaemia show for developing iso-antibodies against incompatible Rh blood group antigens, make it likely that this kind of carefully selected transfusion (which inevitably means introducing foreign Rh groups) could not be given more than once or twice to any given patient, and has therefore very little practical value.

In most patients the presence of non-specific auto-antibodies makes the cross matching of blood exceedingly difficult and it may be impossible to find blood that is wholly compatible. This difficulty combined with the very transient benefit and the increased load of haemoglobin breakdown products from increased corpuscular destruction that/

that is likely to follow the giving of blood sometimes causes doubt about the wisdom of transfusion. Fortunately in practice, if blood, homologous in respect of the ABO and Rh blood groups, is given, and the best possible cross match with the patient used, this seldom causes severe transfusion reactions in these patients and is frequently life saving.

Steroid therapy

Since the introduction of adrenocortico-steroids, there has been no question that they form the main line of treatment in patients with autoimmune haemolytic anaemia. The first enthusiastic reports came from Dameshek (1950) and Gardner (1950); the first results in Britain (M.R.C. Haematology Panel 1952, 1953) were less convincing, almost certainly because smaller doses were employed. The report of Dameshek (1952), in which 14 of his 22 patients so far treated had shown complete haematological remissions, indicated that this was a major therapeutic development; Dameshek described that suppression of haemolysis could almost always be achieved but this might require cortisone or adrenocorticotrophic hormone (ACTH) in doses of 300 mg. per day. Further experience has shown that in the idiopathic disorder, complete and frequently rapid relief from anaemia can be expected in 70 to 90 per cent of patients with the warm type of antibody (Dameshek and Komninos 1956, Dausset and Colombani 1959). In the symptomatic disease results/

results are less good and when cold antibodies are present (Dausset and Colombani 1959) remission is much less frequent.

The mode of action of adrenocorticosteroids is not definitely established. The evidence for interference with the reaction between erythrocyte antigen and antibody is inconclusive. A number of clinical studies have, however, indicated diminution in the strength of the Coombs' test (Evans 1955, Dameshek and Komninos 1956, and Pisciotta and Hinz 1957), and disappearance of antibody from the serum in patients obtaining remission from steroid therapy, indicating that inhibition of antibody production might be one important effect. In spite of good clinical and haematological response, however, the direct antiglobulin test seldom becomes negative.

There are conflicting views about the relative merits of different steroid preparations. De Gruchy (1954) and Dausset and Colombani (1959) found ACTH to be effective in occasional patients in whom an oral preparation had failed, but this has not been the experience of Wintrobe (1961). Dameshek and Komninos (1956) were impressed with prednisone in comparative studies with cortisone and hydrocortisone, and Dausset and Colombani (1959) regarded prednisone as the best drug for use in the average patient. Particularly in view of its low salt retaining effect, prednisone now seems to be the drug that is most widely used; according to Dacie (1962) prednisolone has no additional advantage and there/

there is as yet insufficient evidence to suggest that the newer analogues of cortisone (methylprednisolone, triamcinolone, dexamethasone) are in any way superior to prednisone. Dacie (1962) quotes from a recent review by Horster (1961) of 167 patients with idiopathic autoimmune haemolytic anaemia; 6.8% were "cured", 31.7% were still in remission twelve months after stopping treatment, 51% were in remission only during treatment and 17% were not benefited.

The data from Horster highlight the recurring therapeutic dilemma. In little more than one third of his large series of patients was lasting remission from steroid therapy achieved. In a large proportion of the remainder the question of splenectomy would arise, either because no remission had been achieved or because it was not possible to subdue the haemolytic process without maintaining the steroid therapy in doses that would inevitably cause side effects. It seems from experience that maintenance doses of prednisone exceeding 10 mg. per day in the adult cannot be contemplated without misgiving.

Splenectomy

Splenectomy was probably the earliest form of treatment for autoimmune haemolytic anaemia, the first report of a successful result being ascribed to Micheli (1911).

Isolated accounts followed but it was Dameshek (1943) who first gave some index of the remission rate when he described/

described good results in 10 of his 18 patients. Prior to the introduction of steroids this was the only treatment and it was considered that splenectomy was beneficial in approximately 50% of cases (Welch and Dameshek 1950). According to Young, Miller and Swisher (1957), two thirds of their 18 patients did well; Crosby and Rappaport (1957) had good responses in 10 of their 27 patients; Dausset and Colombani (1959) undertook splenectomy in 31 patients of whom 8 recovered and these authors indicate that a good result from splenectomy is less likely when cold antibodies are present than with the warm variety; Richmond (1961) found a good result from operation in 6 out of 22 patients treated by splenectomy in Edinburgh, and Dacie (1962) obtained clinical cure in 7 of his 26 patients so treated, adding that a "fair" result was obtained in 4 others.

When steroid therapy has failed completely or has failed to sustain remission in acceptable doses, then splenectomy is usually undertaken. It seems from the foregoing that substantial benefit or clinical cure occurs in a variable proportion of patients but can probably be expected in only about one third. It is a general experience that an additional number will get some reduction in the haemolytic process, and their haemoglobin value may stabilise at a subnormal level where red cell destruction and marrow activity can continue in equilibrium; alternatively this may be achieved by a smaller dose of steroids than/

than was required before operation. Chertkow and Dacie (1956) drew attention to the lack of any clinical criteria from which the outcome of splenectomy could be predicted, but reference has already been made (p.47) to the assessment of the role of the spleen in the haemolytic process by measuring the relative splenic uptake of chromium-51 labelled red cells. However, even when the latter procedure suggests that splenectomy may not be helpful, the problem of dealing with the patient, with relentless haemolytic disease uncontrolled by steroid therapy, still remains and usually surgery is always recommended in the last resort.

The possible role of the spleen in autoimmune haemolytic anaemia has been the subject of much investigation. That the spleen is an important source of antibody is suggested by the observation of Wright, Dodd, Bouroncle, Doan and Zollinger (1951) who found that in patients with autoimmune haemolytic anaemia there was a higher titre of antibody in the spleen substance than in the peripheral blood. Wagley, Shen, Gardner and Castle (1948) and Jandl, Richardson, Jones and Castle (1957) to some extent supported this by observing that red cells leaving the spleen in the splenic vein tended to be more heavily sensitised than cells in peripheral venous blood. Studies in patients with autoimmune haemolytic anaemia using ^{51}Cr labelled red cells and experiments in normal subjects (Jandl, Richardson, Jones and Castle 1957, and Mollison and Hughes-Jones 1958) have clearly/

clearly shown that the spleen removes cells from the circulation that are coated with incomplete antibody, and in normal recipients of sensitised cells this action is extremely rapid; on the other hand coarsely agglutinated cells are taken out of the circulation mainly in other areas, particularly the liver.

From the earliest definite recognition of autoimmune haemolytic anaemia (Dameshek and Schwartz 1938, 1940), the existence of a fulminating type of disease has been known. At the present time there is still a small proportion of patients who, despite adequate steroid therapy and splenectomy, persist with a violent haemolytic process, who require frequent transfusion for survival and who proceed to a fatal termination which is often accelerated by the side effects of treatment. An entirely new approach has recently been proposed for the management of this group.

Antineoplastic agents

In 1951 Dameshek reported the use of intravenous nitrogen mustard therapy in four patients with autoimmune haemolytic anaemia with a view to suppressing antibody production; the desired result was achieved in the first patient treated, the antibody titre fell and a remission persisting for four years followed. A similar therapeutic attack was tried with encouraging results by Wang, Bowman and Tocantins (1954) and Tocantins and Wang (1956), when they used intravenously administered radioactive gold. Following evidence that 6-mercaptopurine/

6-mercaptopurine and thioguanine were potent inhibitors of immunity responses in experimental animals (Schwartz, Stack and Dameshek 1958, Schwartz and Dameshek 1959), Dameshek and Schwartz (1960) described the use of these agents in the treatment of autoimmune haemolytic anaemia. The later report of these authors (Dameshek and Schwartz 1962) gives some ground for optimism in that 14 patients were treated with 6-mercaptopurine or thioguanine or both and 9 showed a response; in some of these, haematological remission was sustained for periods up to fourteen months, even after withdrawal of antimetabolite therapy. The remarkable effect of this type of treatment, using new antineoplastic preparations, will be described in Case 3 of this series. This patient had a fulminating haemolytic process in the presence of severe thrombocytopenia.

3. 5. Summary of the management of individual patients in the present series.

The main lines of treatment that have been employed in the 23 patients with autoimmune haemolytic anaemia, studied in the present series, are summarised in Table 7.

Additional details are given in the brief accounts of individual patients which follows:-

Case 1. M.B. 73 years; An elderly lady with the "cold haemagglutinin syndrome". Steroid therapy was tried intermittently in 1956 without significant benefit. She maintained moderately good health since that time without further/

TABLE 7

SUMMARY OF MAIN FORMS OF TREATMENT IN PATIENTS WITH AUTOIMMUNE HAEMOLYTIC ANAEMIA

Patient	1	2	3 *	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19*	20	21	22	23
	M.B.	H.D.	M.F.	J.F.	C.H.	I.H.	H.L.	M.L.	N.L.	E.L.	I.L.	M.M.	H.O.	J.R.	C.S.	I.S.	A.B.	I.E.	G.D.	D.M.	I.M.	J.S.	E.W.
Blood Transfusion	0	+	+	+	+	+	+	+	+	0	0	+	0	0	+	+	0	+	0	+	+	+	+
Steroid Therapy	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Splenectomy	0	+	+	+	+	0	+	+	0	0	0	0	0	0	+	0	0	0	+	+	+	0	0

* Case 3 also received mediastinal irradiation, a cytotoxic drug and an antimetabolite (p.113).
Case 19 was also treated by splenic irradiation.

further treatment.

Case 2. H.D. 61 years: A male patient who presented in 1955. After an initial response to transfusion and cortisone therapy, remission could not be sustained and splenectomy was undertaken three months later. Operation controlled haemolysis for approximately twelve months after which time a chronic partially compensated haemolytic process returned. Steroid therapy was not reintroduced and the patient died after a further three years following myocardial infarction.

Case 3. M.F. 35 years: Fulminating haemolytic anaemia developed in this young female patient due to a cold type antibody while she was receiving steroid therapy for relapse of idiopathic thrombocytopenic purpura, previously successfully treated by splenectomy in 1956. Control of the haemolytic process could not be achieved with large doses of prednisone or cortisone and copious blood transfusion was necessary for survival. Improvement was eventually achieved after irradiation and the use of antineoplastic drugs (p. 113).

Case 4. J.F. 9 months: An infant girl in whom haemolysis could only be controlled by large doses of steroids; repeated attempts to reduce the prednisolone, to a level which would allow growth to proceed, were made over a period of five months. Splenectomy was then undertaken with only transitory improvement in the disease and the child died four/

four months later during an attempt at exchange blood transfusion.

Case 5. C.H. 46 years: A severe haemolytic anaemia which was not controlled on steroid therapy and for which blood transfusion was required. After six weeks on prednisone (see p. 94 for complications) splenectomy was undertaken. The haematological findings in the next three months suggested that this had been unsuccessful, but coincident with gradual withdrawal of prednisone, the haemoglobin level improved and the reticulocyte count fell; the patient remains well twelve months later with compensated haemolysis, the haemoglobin level being in the range 80-90% and the reticulocyte count 2-4%.

Case 6. I.H. 72 years: An elderly female patient who required blood transfusion for survival and in whom prednisone was ineffective. Splenectomy could not be considered because of ischaemic heart disease and congestive cardiac failure, and she died of the latter two months after diagnosis.

Case 7. H.L. 24 years: A girl who presented after a haemolytic crisis due to a cold type antibody in 1955. Multiple blood transfusions were required initially and then cortisone produced a partial remission. After three months splenectomy was undertaken without further influence on the haematological findings. Since then (now seven years) the patient has remained in comparatively good health.

A chronic haemolytic process persists and the two further haemolytic crises which she has had have been dramatically responsive to the giving of prednisone (p.108). Two normal pregnancies have been supervised during the course of her illness.

Case 8. M.L. 71 years: The chronic haemolytic process in this elderly woman was discovered coincidentally during examination for a respiratory infection. The initial response to prednisone was excellent but withdrawal to a maintenance dosage produced relapse. A further temporary remission followed splenectomy but the patient died five months after diagnosis of abdominal infection, believed to be caused by steroid treatment (p.94).

Case 9. N.L. 31 years: An acute haemolytic process following throat and skin infection. Remission followed transfusion and steroid therapy. The patient remains well with normal haematological findings and negative direct Coombs' test nine months after the initial episode.

Case 10. E.L. 48 years: A sustained remission followed the use of prednisone alone in this patient. She has persistently normal haematological and serological findings after seven years and is clinically well.

Case 11. I.L. 76 years: An elderly lady whose illness has now been followed for six years. The haemolytic process runs a chronic relapsing course; three exacerbations apparently associated with urinary tract infection have shown/

shown a satisfactory response to prednisone. Steroid therapy is not given on a maintenance basis.

Case 12. M.M. 48 years: A fulminating haemolytic process due to a cold non γ -globulin type of antibody in a female patient. Frequent blood transfusions and large doses of prednisone (to 200 mg. per day) were used. The illness was complicated by acute renal failure, phlebothrombosis and pulmonary embolism, and the patient finally died of staphylococcal pyaemia after one month, before splenectomy could be undertaken.

Case 13. H.O. 65 years: The cold haemagglutinin syndrome in an elderly male patient. Anaemia is not severe and he remains in moderately good health through avoidance of cold. Small doses of prednisone have been used intermittently since 1957 without definite benefit.

Case 14. J. R. 50 years: This male patient had an acute haemolytic anaemia due to a warm type antibody. There has been a complete remission on steroid therapy which is persisting after one year; the dose of prednisone has been less than 10 mg. per day for eight months and is now 2.5 mg. daily.

Case 15. C.S. 63 years: A chronic haemolytic process which had probably been present for fourteen months at the time of diagnosis. Transfusions had been found to have only transitory effects on the haemoglobin level. Prednisone was given for six weeks after which time splenectomy was/

was undertaken. The latter had no effect and the patient died in congestive cardiac failure after a further nine months, following a series of respiratory infections which had probably been aggravated by persistent prednisone treatment.

Case 16. I.S. 27 years: This is the only patient in the series shown definitely to have a specific auto-antibody (anti-c). She presented after a severe haemolytic crisis. Transfusion and steroid therapy induced a complete remission. The direct Coombs' test was found to be negative three months after diagnosis, at the time steroid therapy was withdrawn, and the patient remains in perfect health in the five years since then.

Case 17. A.B. 52 years: This male patient who has lymphosarcoma presented with gross splenomegaly. He has been treated with betamethasone and has been in sustained remission, with marked regression of spleen size, for twelve months. He is maintained on Betnelan, 2 mg. daily.

Case 18. I.E. 32 years: This girl with disseminated lupus erythematosus was first seen in remission but she had had episodes of haemolysis in the previous twelve months which had responded to transfusion and the giving of prednisone. She was maintained on a prednisone dosage of 10 mg. daily for twelve months, 7.5 mg. daily for four months and in the eighteen months since then on 5 mg. daily. Complete haematological remission continues.

Case/

Case 19. G.D. 62 years: A patient with lymphosarcoma showing a cold haemolysin of Donath-Landsteiner type. He has received prednisone therapy for six months and has had a course of splenic irradiation without significant benefit.

Case 20. D.M. 64 years: This man had symptomatic haemolytic anaemia in association with giant follicle lymphoma. No improvement followed steroid therapy and on the basis of studies with chromium-51, splenectomy was undertaken. Although operation produced no demonstrable improvement in red cell survival (p. 98) the patient is still moderately well after two years; the haemoglobin level is in the region of 70% without recourse to prednisone.

Case 21. I.M. 4 months: A male child with "familial haemophagocytic reticulosis" who showed no response to splenectomy, exchange transfusion or prednisone therapy and died five weeks after diagnosis (p. 101).

Case 22. J.S. 49 years: A mild haemolytic process, no splenomegaly and a hypoplastic bone marrow infiltrated by lymphocytes were the main features of this patient. Frequent blood transfusions were required and prednisone therapy had no effect on the transfusion requirement. He died of the primary disease three months after diagnosis.

Case 23. E.W. 30 years: This young woman with disseminated lupus erythematosus gave a long history of arthralgia and two years' history of anaemia at the time of diagnosis in 1956. She required blood transfusion for the correction of/

of acute anaemia at the onset. She has remained in complete or near complete remission for the last seven years on prednisone therapy, and currently receives 5 mg. per day.

An analysis of the effects of the various forms of treatment, the selection of patients for splenectomy, the handling of fulminant haemolytic disease that defies orthodox measures, and the natural history of disease as seen in the foregoing 23 patients, is considered in the sections that follow.

3. 6. The effect of blood transfusion.

In no case can it be stated that blood transfusion alone induced remission in the haemolytic process. Despite the possibility of transfusion reactions in patients with autoimmune haemolytic anaemia to which reference has already been made, it is considered that this is much less important than leaving a patient in a hypoxic state from persisting severe anaemia, and it has been a policy in the present series to keep the haemoglobin level above 50% wherever possible. Accordingly, 16 patients of the 23 studied have required transfusion at one time or another during their management. In Cases 2, 5, 7, 9, 16, 18, 20 and 23, the giving of blood was only necessary to correct the initial severe anaemia, and in Cases 2, 5, 7 and 20, during the period of splenectomy. In the others (Cases 3, 4, 6, 8, 12, 15, 21 and 22), recurrent transfusion was necessary and in Cases

3, 4, 12 and 21 it was believed to be necessary for survival. Severe transfusion reactions were not seen in any of the patients, although minor febrile reactions were common; an urticarial response occurred during one transfusion in Case 3 but this responded promptly to an antihistamine preparation and several litres of blood were given to this patient subsequently without difficulty.

Cases 3 and 12 required unusually large volumes of blood; the first received 38 litres during the first forty-five days of treatment, and the second 9.5 litres in seven days. Both of these patients showed haemoglobinaemia, methaemalbuminaemia and haemoglobinuria and in both, bile appeared in the urine, and the serum bilirubin rose to levels of 10-15 mg./100 ml. It might be argued that the development of an obstructive type of jaundice was due to the large pigment load which the liver was required to handle; however, liver inefficiency might equally have been due to hypoxia from the recurring severe anaemia, and the evidences of intravascular haemoglobin catabolism indicate the fulminating nature of the haemolytic process in these two patients.

In all the patients suffering from autoimmune haemolytic anaemia in this group, the effect of transfusion was short lived. One constant effect was suppression of reticulocytosis; this occurred promptly on raising the haemoglobin level and is well illustrated by Case 6 (Fig. 14). Since this/

this patient had also suffered some bleeding from a hiatus hernia, the disappearance of reticulocytosis early in the course of investigation led to some diagnostic confusion.

Because of the general belief that transfusion may be hazardous in patients with autoimmune haemolytic anaemia, there is a tendency to cautiously give small transfusions. The consequence of too timid management in a severe haemolytic process is illustrated by Case 12 in Fig. 14. In this patient the haemoglobin level fell to 25% and was again at this level five days later despite the giving of 1 litre of blood on each day in the meantime, and despite the administration of large doses of prednisone. The giving of 4.5 litres of blood in one transfusion (Fig. 14) then had a very satisfactory effect; the haemoglobin level was raised to 65% and it stayed in this range for several days thereafter.

The impression has been gained in this (Case 12) and in other severely anaemic patients that the giving of large amounts of blood is the correct procedure in the initial illness. Not only does a large transfusion raise the haemoglobin to safe levels, but also it appears to temporarily reduce the rate of blood destruction and this might tide the patient over a critical period until other measures such as steroid therapy become effective. It is not known how such reduction in the severity of haemolysis arises; it is possible that the transfusion of large numbers of healthy/

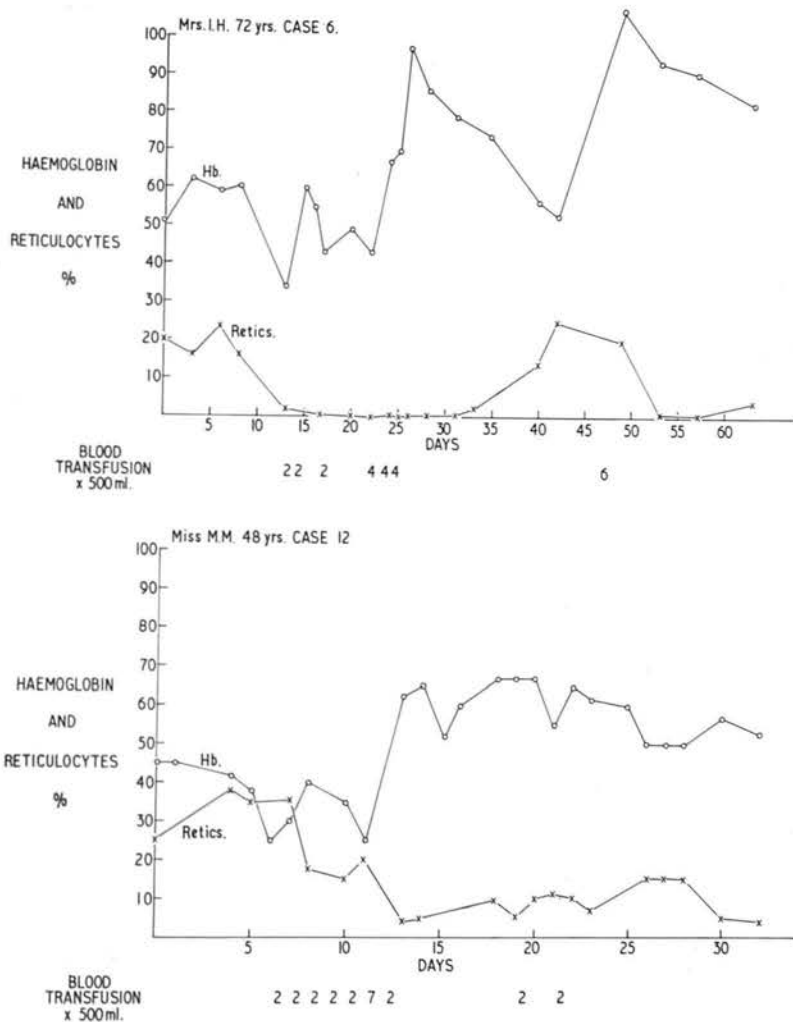


Fig. 14

Two patients with autoimmune haemolytic anaemia who required blood transfusion. Both illustrate the suppressive effect of transfusion on reticulocytosis; Case 12 also illustrates that a large transfusion may temporarily suppress the severity of the haemolytic process.

healthy cells "mops up" free circulating antibody, or alternatively that the antibody coating red cells may be redistributed from heavily sensitised cells to donor cells; in either event some improvement would be expected to follow and there is experimental evidence in favour of the latter possibility. Both Mollison (1959) and Evans, Bingham and Boehni (1961) have shown that incomplete Rhesus antibody can be transferred in vitro from sensitised cells to normal cells that are incubated with them.

3. 7. The effect of steroid therapy.

All 23 patients in the present series have received steroid therapy during the course of their illness. Cases 1, 2, 3, and 7 have received cortisone; every patient given steroids since 1956 has received prednisone as the main preparation, except Case 3 in whom cortisone was also tried, Case 4 who was treated with prednisolone and Case 17 who received betamethasone.

The clinical effects of steroid therapy may be summarised as follows:

(i) No significant benefit, 11 patients (Cases 1, 3, 5, 6, 12, 13, 15, 19, 20, 21 and 22). Of these, 6 had a cold type antibody, 4 a warm type; in Case 21, as has been stated, a positive direct Coombs' test result is not available.

(ii) Transitory benefit, 4 patients (Cases 2, 4, 7 and/

and 8). Of these, 3 had a warm type of antibody and 1 a cold type.

(iii) Significant benefit in that steroid therapy formed the main line of treatment, 8 patients (Cases 9, 10, 11, 14, 16, 17, 18 and 23). Of these, 5 had a warm type of antibody and 3 a cold type.

The experience of response in respect of the type of antibody is slightly different from that of others (p. 76) in that only 8 of the 12 patients (67%), with a warm type of antibody, showed a sustained or a transitory response to steroid treatment.

In those patients who showed remission from steroid therapy, three patterns of response were noted. These are illustrated in Fig. 15. In the first pattern (Case 11), there was a gradual rise of haemoglobin level and fall in reticulocyte count, but the steroid therapy had to be continued in a small dose to sustain the remission. In the second pattern (Case 7) the same satisfactory effect was observed. After reaching normal peripheral blood values the steroid therapy could be completely withdrawn and remission was sustained either indefinitely (seven years in Case 10) or for many months. In the third pattern (Case 2) the initial response was equally good but the steroid therapy could not be withdrawn to a level which could be contemplated for long term management without inducing relapse. This problem is even better illustrated in Fig. 16 which/

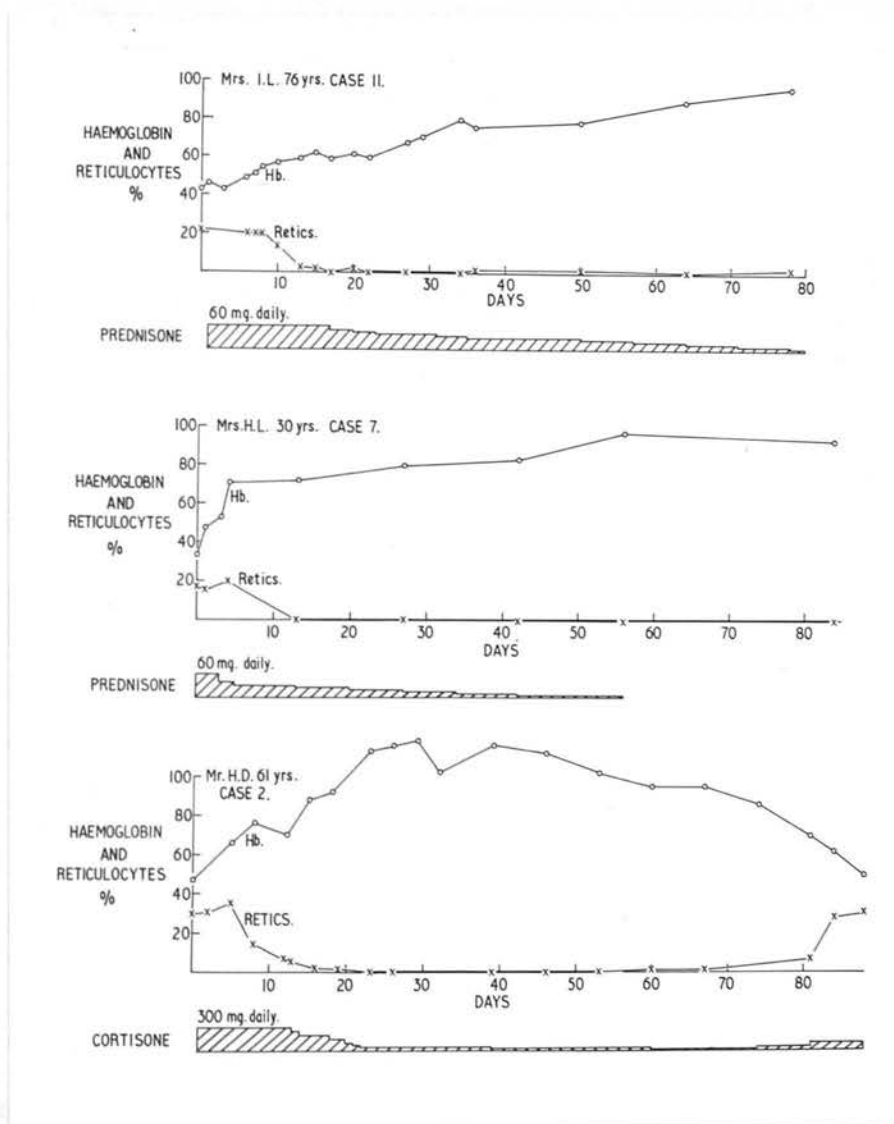


Fig. 15

The patterns of response to steroid therapy in autoimmune haemolytic anaemia. Case 11 required a small maintenance dose of steroid therapy to sustain remission; in Case 7 remission continued after withdrawal of therapy, while in Case 2 anaemia is seen to return when the dose of steroids is reduced to an acceptable maintenance level.

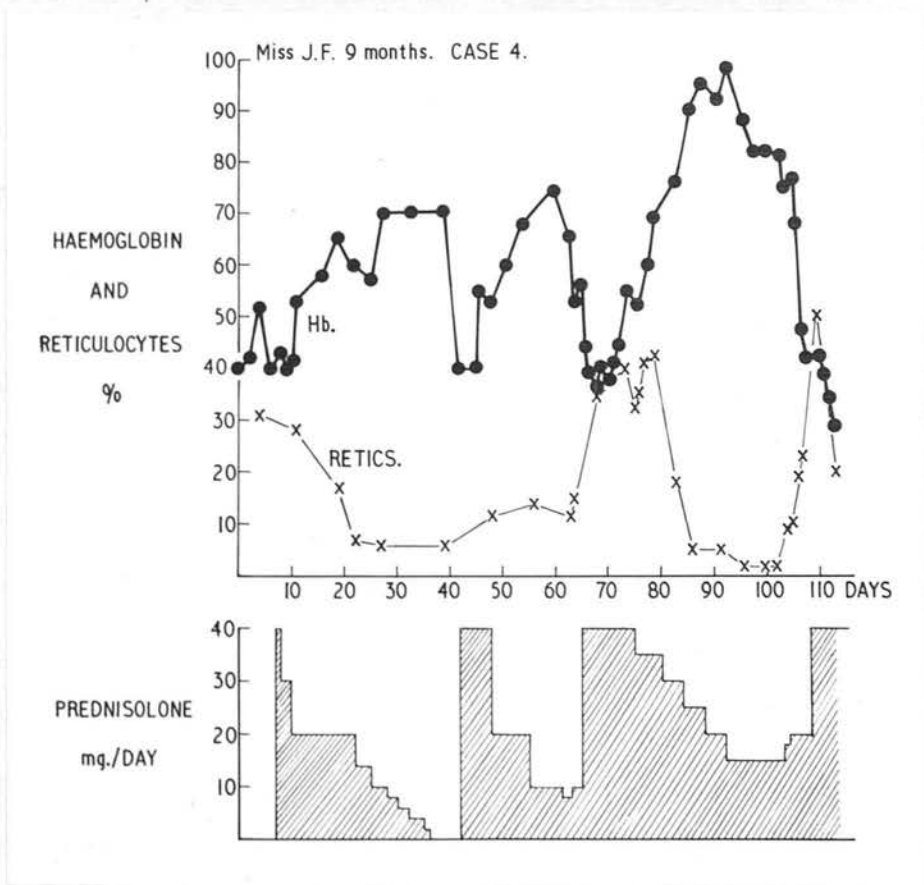


Fig. 16

An infant with autoimmune haemolytic anaemia who showed dramatic response to the giving of prednisolone; each attempt to withdraw steroid therapy to a maintenance level caused return of severe anaemia.

which shows the course of a child aged nine months (Case 4). In this patient the blood picture could be corrected rapidly with the giving of high doses of prednisolone, but on repeated attempts to reduce the dose of the steroid to a level which would allow growth to proceed, severe haemolysis returned.

Figs. 15 and 16 illustrate the very rapid improvement that is often seen when steroids are exhibited. This was particularly evident in Case 7 (Fig. 15) in whom the haemoglobin level rose from 34% to 71% in four days indicating the tremendous compensatory activity of the healthy bone marrow during haemolysis. Another point which is worthy of comment is the very prompt suppression of reticulocytosis (similar to that found after blood transfusion) when steroids are given; this is a useful indication of the effectiveness of treatment, since a falling reticulocyte count sometimes precedes significant change in the haemoglobin level.

Complications.

Except in Cases 1 and 13 (the two patients with the cold haemagglutinin syndrom), steroids have always been used in large doses in the first instance, either 300 mg. of cortisone or 60 mg. of prednisone (the exceptions are 40 mg. of prednisolone in Case 4 and 8 mg. of betamethasone in Case 17) being given. These amounts were not continued for longer than three weeks (except in Case 3), being gradually/

gradually reduced after this time or earlier if remission had occurred. Perhaps because of the high doses that are employed some side effects from treatment are inevitable, but the frequency and severity of these are worthy of mention:

(i) Fluid retention. Cases 2, 3 and 12 developed marked oedema while receiving steroid therapy. In Cases 2 and 3 cortisone was being given at the time. In Case 12 the complication arose during the administration of prednisone; anasarca developed in this patient but acute renal failure was also implicated.

(ii) Perforation of a viscus. Case 5 perforated an acute duodenal ulcer while being treated with prednisone; although this had undoubtedly occurred on 7.9.61, the clinical features were so atypical and the inflammatory response so mild that the diagnosis was not made until the abdomen was opened for splenectomy on 26.9.61. Case 12 developed low grade peritonitis from rupture of a pyometra while on prednisone. This, which followed an unsuccessful splenectomy, and the subsequent laparotomy are believed to have been the immediate reasons for the patient's death.

(iii) Burst abdomen following surgery. This complicated the course of two patients, Case 4 who was receiving prednisolone at the time and Case 15 who was on prednisone. Both patients were convalescing from splenectomy.

(iv)/

(iv) Miscellaneous complications. Case 12 developed extensive phlebothrombosis which was succeeded by a pulmonary embolism; this particular patient went on to die from staphylococcal pyaemia, and it seems reasonable to believe that this might also have been aggravated by prednisone therapy. The terminal illness of another patient, Case 15, was also influenced by (recurrent respiratory) infection. Case 8 who had been showing mild features of senile dementia became frankly psychotic.

In summary, 7 (30%) of this series of 23 patients with autoimmune haemolytic anaemia developed one or more major complications at some stage in their illness, apparently directly due to their management with a steroid preparation. Reference will be made in the next chapter on symptomatic non-immune haemolytic anaemia to a further patient who developed severe diabetes mellitus while taking prednisone (p. 144). This very high incidence of side effects in these patients with haemolytic disease and the illustration that long continued steroid therapy might even interfere with the patient's successful management by splenectomy (Cases 4 and 15), influence one to use the smallest possible dose for the shortest possible time.

3. 8. The selection of patients for splenectomy and the results of operation. The "Spleen Number".

Nine patients from the whole group have had a splenectomy undertaken. In Case 3 the spleen had been removed before/

before the haemolytic process started (p. 113), in Case 21 operation was undertaken at an early stage because of previous experience with two siblings who had had the same disease; in the other seven patients splenectomy was recommended because of failure to control the haemolytic process with steroid treatment. It should be stated that Cases 6 and 12 would also have proceeded to surgery had their condition allowed.

In three patient (Cases 3, 15 and 21) there was no evidence of any beneficial effect from operation; in another two (Cases 4 and 8) improvement was so short lived as to be of no value. Of the other four patients, none has shown clinical cure but Case 5 is significantly better, maintaining a high haemoglobin level after fifteen months; Cases 2, 7 and 20 became stabilised at a moderate level of anaemia and have remained in fair health for two to seven years; in Case 7 the haemolytic crises that did occur after operation were more responsive to steroid therapy than previously.

Body surface measurements of radioactivity over liver, spleen and praecordium, during the course of estimation of red cell survival with chromium-51, were undertaken in 12 of the patients with autoimmune haemolytic anaemia. The results in terms of spleen sequestration index and spleen/liver ratio at $T_{1/2}^{51}\text{Cr}$ (p. 48) are set out in Table 8.

The five patients who were so investigated and went on to splenectomy/

TABLE 8

DATA OBTAINED IN RESPECT OF RED CELL SURVIVAL TIME AND SPLENIC UPTAKE OF RADIOACTIVE LABELLED

RED CELLS IN PATIENTS WITH AUTOIMMUNE HAEMOLYTIC ANAEMIA AT THE TIME OF FIRST DIAGNOSIS

Patient	4	5	8	10	11	12	13	14	15	17	19	20
	*	*	*						*			*
	J.F.	C.H.	M.L.	E.L.	I.L.	M.M.	H.O.	J.R.	C.S.	A.B.	G.D.	D.M.
$T_{1/2}Cr$ in days	11	3	8	4	17	1.1	6	7	15	11.5	16	6.5
Spleen sequestration index	20	150	70	245	60	15	78	300	80	40	125	90
Spleen/liver ratio at $T_{1/2}Cr$	0.85	2.3	2.1	2.8	1.2	1.87	1.68	1.51	1.2	1.86	3.5	6.0

* Patients in whom splenectomy was undertaken.

splenectomy are marked with an asterisk. Of these five patients, red cell survival measurement was repeated after splenectomy in two, Case 20 who had a partial remission and Case 5 who may be described as having a good result; the data obtained in the studies with chromium-51 in these two patients is illustrated in Fig. 17. While Case 5 shows significant reduction in the rate of destruction of red cells, Case 20 shows none and it must be assumed that in the latter patient his better ability to maintain the haemoglobin level in the region of 70% without transfusion and without steroid therapy after operation is due to more effective compensatory activity on the part of the marrow.

Study of Fig. 17 and of the earlier Fig. 10 (p. 55) which was used to describe the determination of the spleen sequestration index and of the spleen/liver ratio at $T_{1/2}Cr$, indicates that neither of these measurements adequately describe the sequestration of ^{51}Cr labelled red cells by the spleen in relation to the other area which has been monitored, the liver. A patient might have a high spleen sequestration index (this value being derived solely from the increment of radioactivity in the spleen area) and at the same time show a high uptake of radioactive labelled erythrocytes in the liver, a factor which would give a low spleen/liver ratio. Alternatively, a patient might have a high spleen/liver ratio and a low spleen sequestration index (Case 8, Table 2). This latter could occur if the patient had/

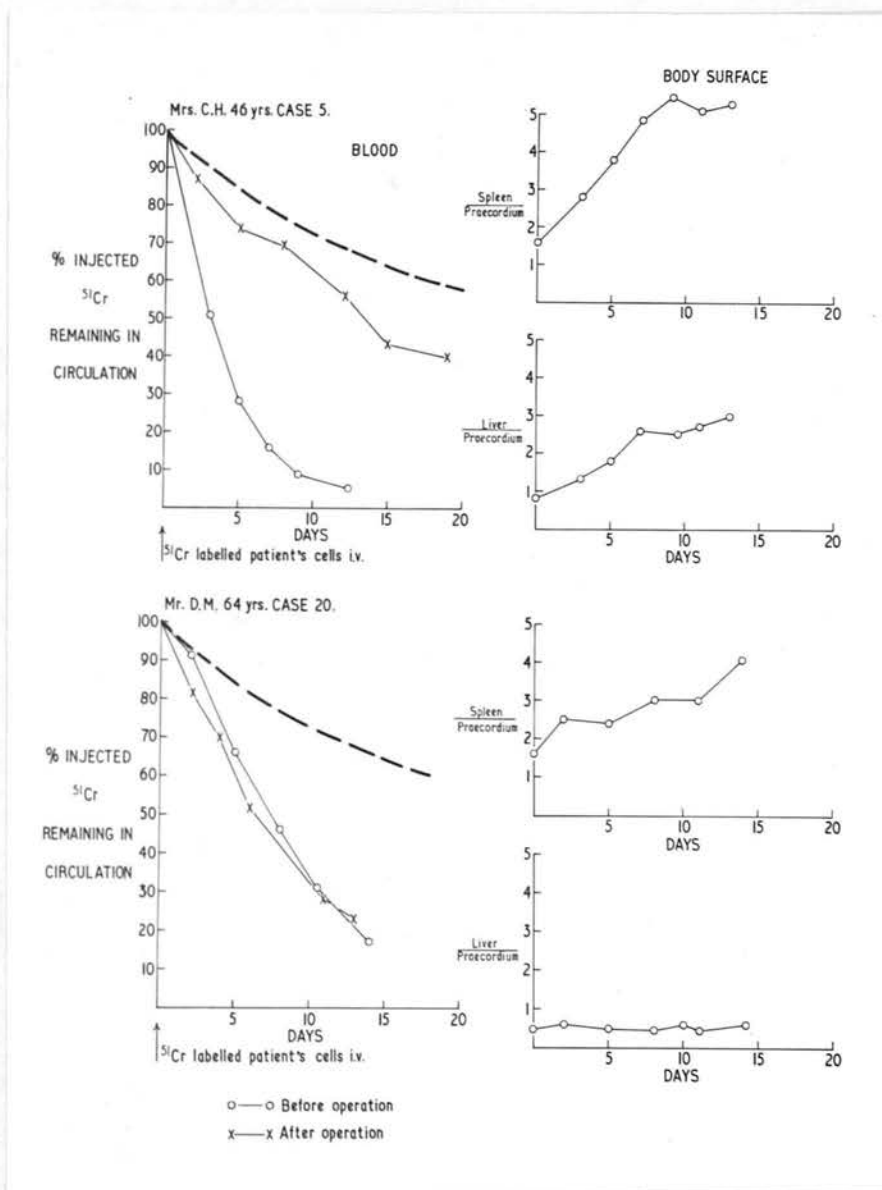


Fig. 17

Red cell survival measurement in two patients with autoimmune haemolytic anaemia before and after splenectomy. Both were expected to show reduction of the haemolytic process on the basis of splenic sequestration measurements (see text); however, while improvement in cell survival is seen in Case 46, none is seen in Case 64.

had a large vascular spleen, causing a high amount of radioactivity in the spleen area in the initial part of the study, but subsequently the spleen showed little increment of radioactivity from sequestration of labelled red cells.

When the patients are classified according to response to splenectomy - "no response", "transitory response", "partial remission" and "good result" - and the data available for the spleen sequestration index and the spleen/liver ratio are studied, no definite pattern of response is seen (Table 9). The spleen/liver ratio in Case 5 (2.3) who had a good result is only marginally better than that in Case 8 (2.1) who had but transitory benefit from operation; the highest spleen/liver ratio is in Case 20 (6.0) but he had only a partial remission and no measurable improvement in red cell survival following removal of the spleen. Reference has already been made to the observation of Goldberg (1960) who found that when the spleen sequestration index exceeded 2.4, a good result from splenectomy could be anticipated. With regard to the spleen sequestration index, there is little difference between the value of 80 in Case 15 who showed no response, 70 in Case 8 who had a transitory response and 90 in Case 20 who had a partial remission. On the other hand, the highest value, 150, is seen in Case 5 who had the best result.

From the foregoing it seems necessary to consider both the increment of radioactivity in the spleen area (the/

TABLE 9

CLASSIFICATION OF PATIENTS WITH AUTOIMMUNE
HAEMOLYTIC ANAEMIA ACCORDING TO RESPONSE TO SPLENECTOMY
SHOWING AVAILABLE BODY SURFACE MEASUREMENTS DURING
STUDIES WITH CHROMIUM-51 LABELLED RED CELLS

Patient	Spleen Sequestration Index (A)	Spleen/Liver Ratio at $T_{1/2}Cr$ (B)	A x B
<u>No response to splenectomy</u>			
Case 3	-	-	-
15	80	1.2	96
21	-	-	-
<u>Transitory response</u>			
Case 4	20	0.85	17
8	70	2.1	147
<u>Partial remission</u>			
Case 2	-	-	-
7	-	-	-
20	90	6.0	540
<u>Good result (but not clinical cure)</u>			
Case 5	150	2.3	345

(the sequestration index, A in Table 9) and the relative amounts of radioactivity in liver and spleen (the spleen/liver ratio, B in Table 9) in deriving a figure which can more adequately evaluate the role of the spleen in the haemolytic process. By the simple expedient of multiplying A and B, this requirement seems to be satisfied and it is seen from Table 9 that in the only two patients for whom data are available, who have had any remission from splenectomy, this multiple exceeds 300, being 540 in Case 20 and 342 in Case 5.

The body surface measurements have been treated in the same way for the patients who did not have a splenectomy and the results are as follows:-

		Spleen Sequestration Index A	Spleen/Liver ratio at $T_{\frac{1}{2}Cr}$ B	A x B
Case 10	E.L.	245	2.8	734
Case 11	I.L.	60	1.2	72
Case 12	M.M.	15	1.87	28
Case 13	H.O.	78	1.68	127
Case 14	J.R.	300	1.51	753
Case 17	A.B.	40	1.86	74
Case 19	G.D.	125	3.5	438

Cases 17 and 19 both had lymphosarcoma with lymphocytic infiltration of the bone marrow, and in view of the multiple factors concerned in the causation of their anaemia and of the/

the varying effects of steroid therapy on the lympho-proliferative disease, they are best considered separately. For the idiopathic cases the two highest figures for the multiple A x B (Cases 10 and 14 in both of whom the figure exceeded 700) were found in patients who had excellent remissions on steroid therapy. Case 12 who had a very low value for A x B had a severe haemolytic disorder which was completely unresponsive to large doses of prednisone.

Further consideration of this multiple of the spleen sequestration index and the spleen/liver ratio at $T_{1/2}Cr$ will follow in the chapter on symptomatic non-immune haemolytic anaemia. For brevity and to avoid confusion, the figure will be referred to in subsequent discussion as the "Spleen Number"; it can be derived from the following equations:-

$$\begin{aligned} \text{Spleen Number} &= (S_t - S_o) \times 100 \times \frac{S_t}{L_t} \\ &= \frac{100 S_t^2 - 100 S_t S_o}{L_t} \end{aligned}$$

where S denotes the radioactivity over the spleen, L the radioactivity over the liver, t is $T_{1/2}Cr$ and o is the first day of study.

3. 9. An unusual family.

Mention has already been made of the familial nature of the illness affecting Case 21. Since the condition appeared to be quite unique and since the main feature was excessive red/

red cell destruction due to an unusual degree of erythrophagocytosis, it is proposed to describe it in more detail.

The title "familial haemophagocytic reticulosis" has already been given by Farquhar and Claireaux (1952) to the disease they had encountered in two older siblings of Case 21. These two infants had both developed anorexia, vomiting, irritability and pallor, associated with hepatic and splenic enlargement, at the age of nine weeks. Peripheral blood examination revealed pancytopenia, progressive anaemia and large numbers of "smear" cells in blood films. The first infant, a male, died at the age of ten weeks. More investigation was possible in the second; as a result the condition was considered to be a symptomatic haemolytic anaemia due to some form of reticulosis and the direct Coombs' test was found to be positive. The second infant showed temporary response to the giving of ACTH but she died at the age of twenty-two weeks.

Autopsy revealed, in both children, a remarkable histiocytic proliferation in reticulo-endothelial tissues which had not been apparent in antemortem marrow smears. These histiocytes showed most striking erythrophagocytosis and less marked ingestion of white cells. The histological picture was not that of Letterer-Siwe's disease, nor was it compatible with histiocytic medullary reticulosis (Marshall 1956).

The/

The third child of the family, a girl, was normal clinically at birth, but peripheral blood examination showed the presence of numerous smear cells; a high percentage of atypical large lymphocytes appeared in the blood between the ages of six and eight weeks, and at ten weeks she developed severe anorexia. The spleen and the liver were at no time palpable but mild anaemia and the atypical lymphocytes were present until the age of eighteen months. At this stage the direct Coombs' test was weakly positive but thereafter became negative again, and after the age of eighteen months the peripheral blood picture became normal. Since then, development has been normal and the child is now apparently healthy, aged ten years.

The fourth child (Case 4 of Farquhar, MacGregor and Richmond 1958) is Case 21 of the present series. He was admitted to hospital aged eighteen weeks, having developed anorexia, irritability and pallor in the previous four weeks. As in the first two children hepatosplenomegaly was evident. The haemoglobin level was 65%, W.B.C. 2,500 of which 64% were large lymphocytes and 16% smear cells, and platelets 32,000. The direct Coombs' test was negative but the serum contained a strong platelet agglutinin. Marrow examination revealed marked normoblastic hyperplasia and the smears also showed many abnormal cells; some of the latter contained abundant clear cytoplasm, others were more primitive with basophilic cytoplasm and frequent nucleoli.

In/

In view of the prominence of erythrophagocytosis in the spleen in the first two children, it was decided to proceed to splenectomy and this was performed six days after admission. The spleen weighed 110 g. and the principal histological feature was marked proliferation of endothelial phagocytes in the pulp sinuses and in smaller numbers in the lymphoid tissue of the Malpighian bodies. The phagocytes were large and most of them contained numerous erythrocytes.

There was no significant improvement after splenectomy and prednisone therapy was therefore started five days after operation; this was continued without apparent benefit until the baby died thirty-five days after admission to hospital. Nine days before this an exchange transfusion, using 1,200 ml. of fresh donor blood was also tried without influencing the course of the disease. Opportunity was, however, taken during this procedure to start a red cell survival measurement; the $T_{1/2}Cr$ of the donor cells was five days.

Autopsy, which had to be confined to the abdomen, revealed changes identical to those found in the first two children; the main feature was the presence in various organs of phagocytic histiocytes. The histiocytes were conspicuous in the bone marrow, lymph nodes, liver, and less so in the intestinal wall, adrenal cortex and pancreas. In the bone marrow and lymph nodes there had been phagocytosis of nucleated cells, but it could not be determined if/

if these were normoblasts or lymphocytes; however, throughout all tissues the outstanding finding, and clearly the most significant from the haematological point of view, was the intense erythrophagocytosis; the phagocytes were large with abundant clear, often vacuolated, cytoplasm and most contained one to ten red cells.

Plates 1 to 6 illustrate the unusual histological features of Case 21 and show the infiltration of liver, spleen and bone marrow by disease and the erythrophagocytosis.

As far as is known, there are no other examples of this familial type of haemophagocytic reticulosis leading to symptomatic autoimmune haemolytic anaemia in the literature. There are, however, many reports of leukaemia and other varieties of reticulosis occurring in several members of a family. Some evidence of abnormality in the father of the children was obtained in this family. When first examined at the time of the death of the second child, his Coombs' test was positive. When re-examined five years later after the death of the fourth child (Case 21) the direct Coombs' test was now negative, the peripheral blood was normal, the marrow was normal and there was no bilirubinaemia or urobilinogenuria; there was, however, shortening of the survival time of the father's red cells, $T_{1/2}Cr$ twenty days. Similar detailed examination of the child's mother revealed no abnormalities, $T_{1/2}Cr$ twenty-seven days.

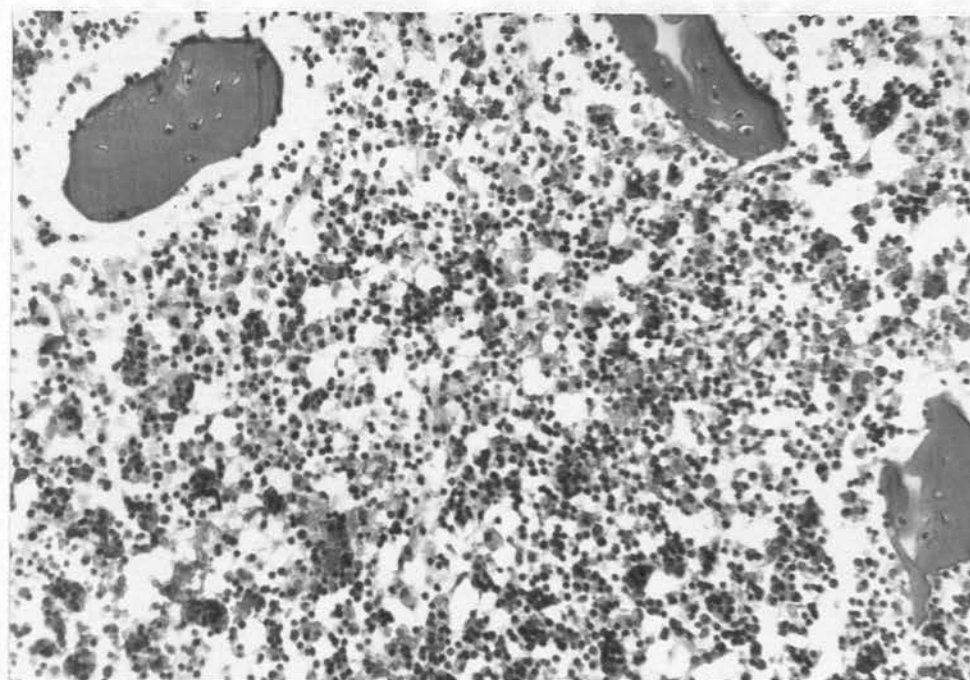


Plate 1

Case 21 Bone marrow. Haematoxylin and eosin x 250.
Infiltration with lymphocytes and histiocytes showing
erythrophagocytosis.

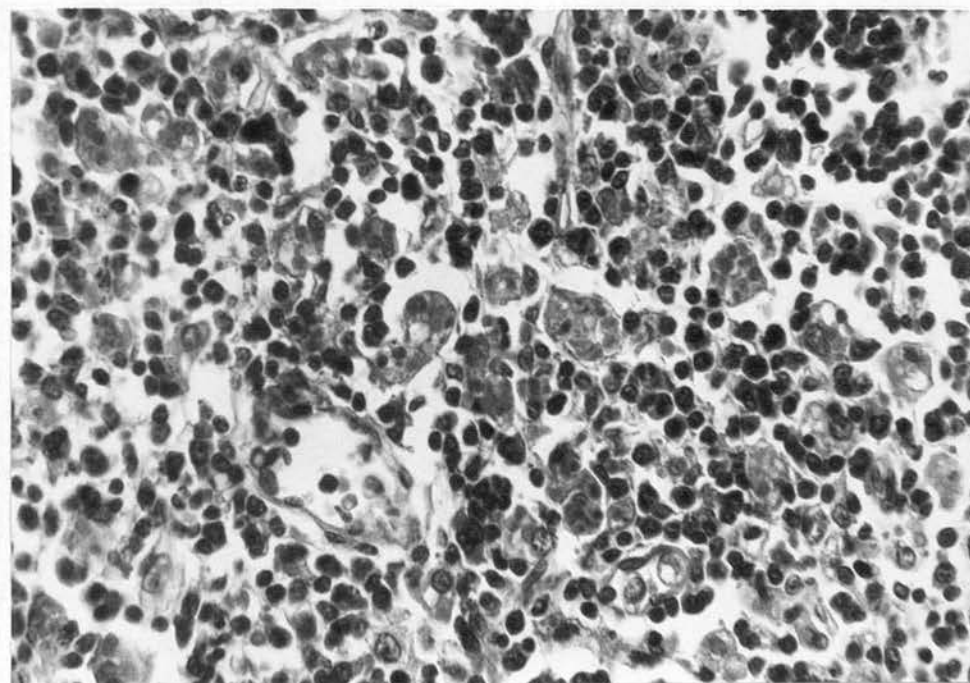


Plate 2

Case 21 Lymph node. Haematoxylin and eosin x 450.
Histiocytic proliferation with erythrophagocytosis.

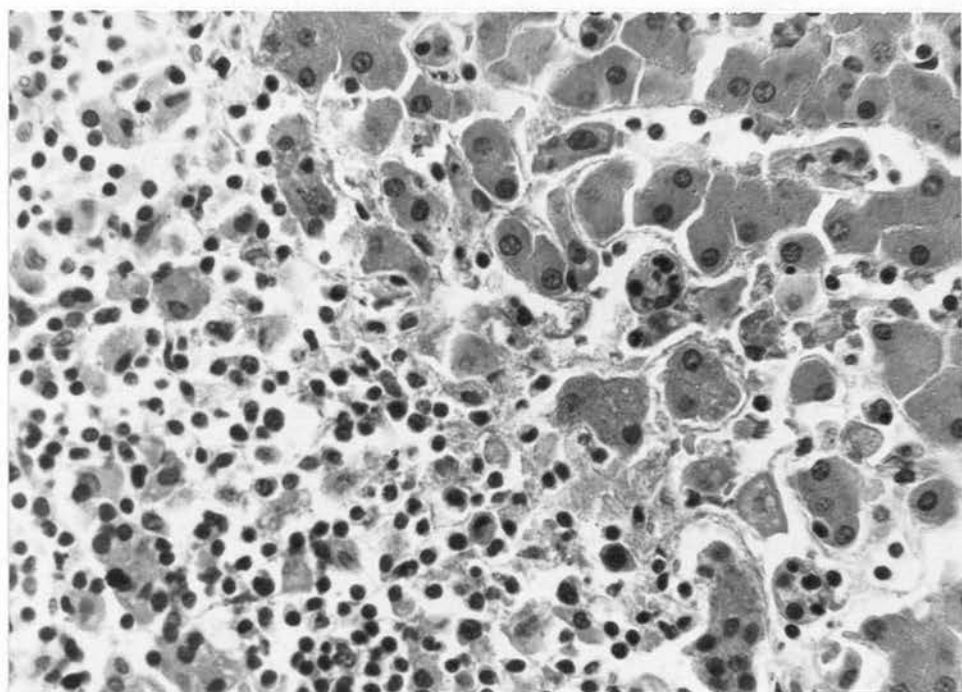


Plate 3

Case 21 Liver. Haematoxylin and eosin x 450.
Infiltration of portal tracts. Histiocytic
proliferation and erythrophagocytosis.

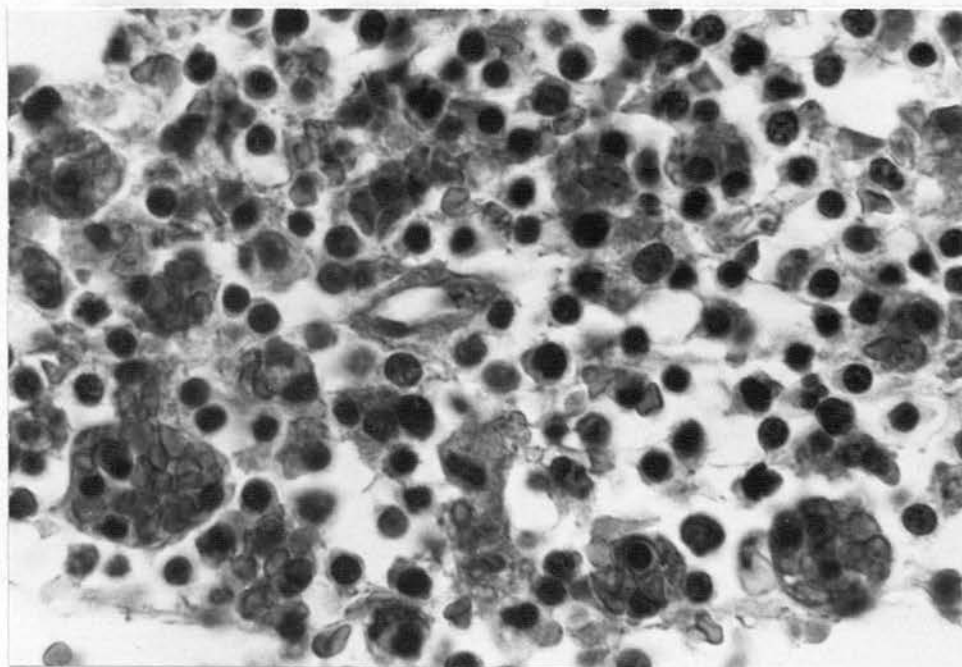


Plate 4

Case 21 Spleen. Haematoxylin and eosin x 1200.
Intense erythrophagocytosis.

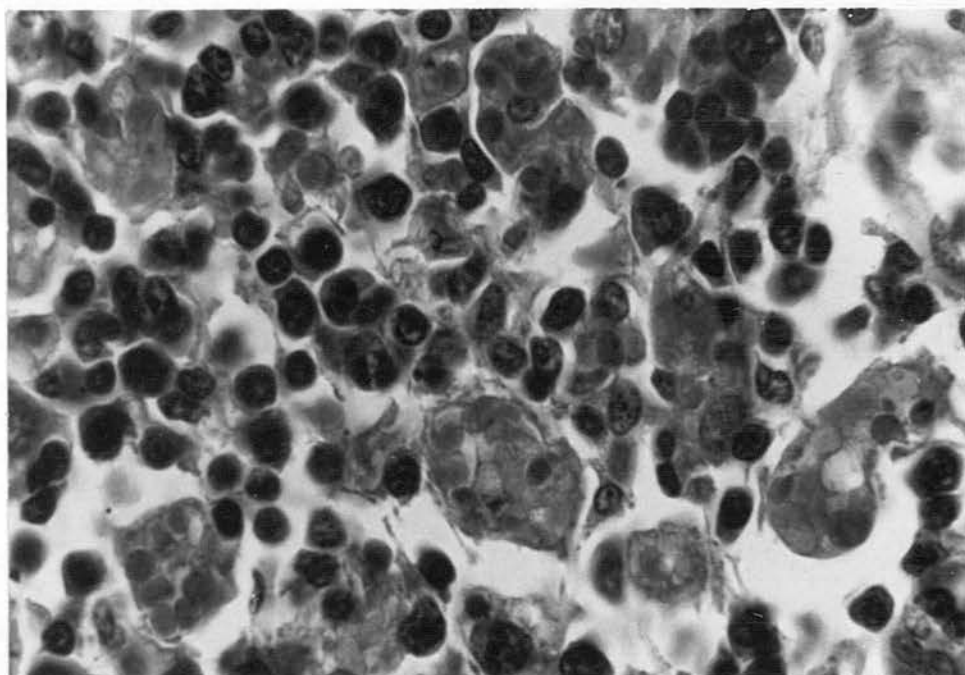


Plate 5

Case 21 Lymph node. Haematoxylin and eosin x 1200.
Erythrophagocytosis and possible leucophagocytosis.

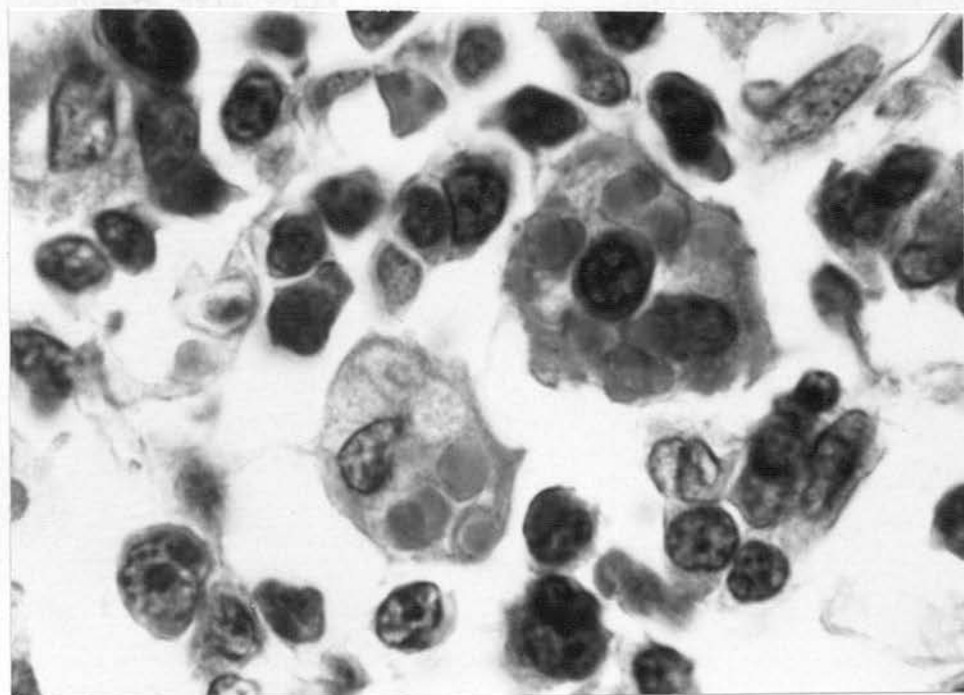


Plate 6

Case 21 Bone marrow. Haematoxylin and eosin x 2000.
Erythrophagocytosis and possible leucophagocytosis.

3. 10. Self limiting idiopathic autoimmune haemolytic anaemia.

In three patients, Cases 9, 10 and 16, a severe haemolytic process has been followed by complete clinical and haematological recovery with conversion of a positive direct Coombs' test to a negative reaction. In all three patients remission followed the giving of prednisone; in Cases 9 and 16 the initial anaemia was so severe that transfusion was also required. The antibody was of cold type in Case 10 and of warm type in Cases 9 and 16. The haemolytic crisis followed a skin and throat infection in Case 9 but no unusual features distinguished the other two; however, the abrupt onset of illness in all three patients and their complete recovery raised the possibility of an infective aetiology.

Although the implication of bacterial and more particularly viral infections in the initiation of autoimmune haemolytic anaemias is not proven, the relationship of virus pneumonia and infectious mononucleosis to a self limiting type of disease is well known; Dacie (1962) also refers to reports of acute haemolytic anaemia following influenza, Coxsackie infection, measles, varicella, encephalitis and herpes simplex.

The course of Case 10 is illustrated in Fig. 18. It will be seen that the Coombs' test became negative two months after the initiation of treatment. This patient has now/

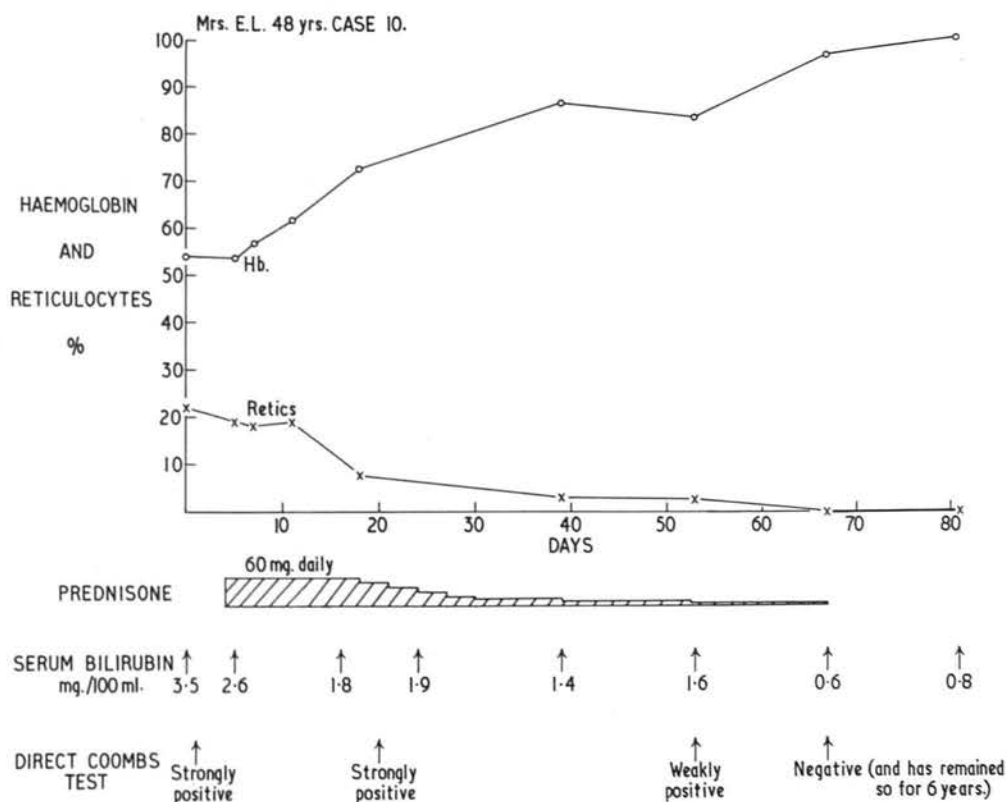


Fig. 18

The course of a patient with self limiting idiopathic autoimmune haemolytic anaemia. A strongly positive direct Coombs' test became negative and the patient has continued in normal health for six years.

now been completely well for seven years. The follow-up period in Case 9 is only nine months but Case 16 is also in perfect health after five years.

3. 11. Chronic relapsing idiopathic autoimmune haemolytic anaemia.

Three patients (Cases 2, 7 and 11) may be described as following a chronic relapsing course extending over many years. Case 2, whose condition was due to a warm type antibody, had an initial response to steroid therapy which was not sustained when the dose was reduced to maintenance levels. Subsequently splenectomy produced a good result for approximately twelve months, after which time a moderately compensated haemolytic process returned. In the next three years, prior to death from myocardial infarction, the haemoglobin level persisted in the range 50-70% and reticulocytosis in the range 5-30%, except for exacerbations of anaemia, the haemoglobin level tending to fall to the 40-50% range after respiratory infections. Case 11, whose disease was also due to a warm type antibody, was not entirely similar. She has been followed for six years and splenectomy has not so far been undertaken. For most of this time the haemoglobin has been at a normal level without the need for treatment, but there have been three well defined haemolytic crises, each of which has been associated with urinary tract infection and each of which has responded satisfactorily to the giving of prednisone.

A patient who illustrates the complications of pregnancy and megaloblastic erythropoiesis.

Case 7 who has followed a chronic relapsing course for more than seven years and has had a positive Coombs' test throughout this time has presented unusual problems and warrants more detailed discussion. The main events are illustrated in Fig. 19.

This patient presented at the age of twenty-four years in the autumn of 1955 following a severe haemolytic crisis. There was no indication of antecedent illness; the initial anaemia was profound, Hb. 21%, reticulocytes 15% and there was mild acholuric jaundice. The spleen could not be palpated. The Coombs' test was strongly positive and further serological studies showed a high titre cold agglutinin to be present. Transfusion was given and cortisone therapy (in a dose of 300 mg. daily) was started. There was indication of suppression of the haemolytic process but the haemoglobin level was never higher than 72% and the lowest reticulocyte counts were in the 5-10% range. Gradual reduction of cortisone produced return of severe anaemia and reticulocytosis, and two months after the initial diagnosis it was decided to proceed to splenectomy. The spleen was not large, weighing only 230 g.; many splenunculi present in the splenic pedicle were also removed.

In the light of present knowledge it might now be expected that little would come of surgery in this type of patient/

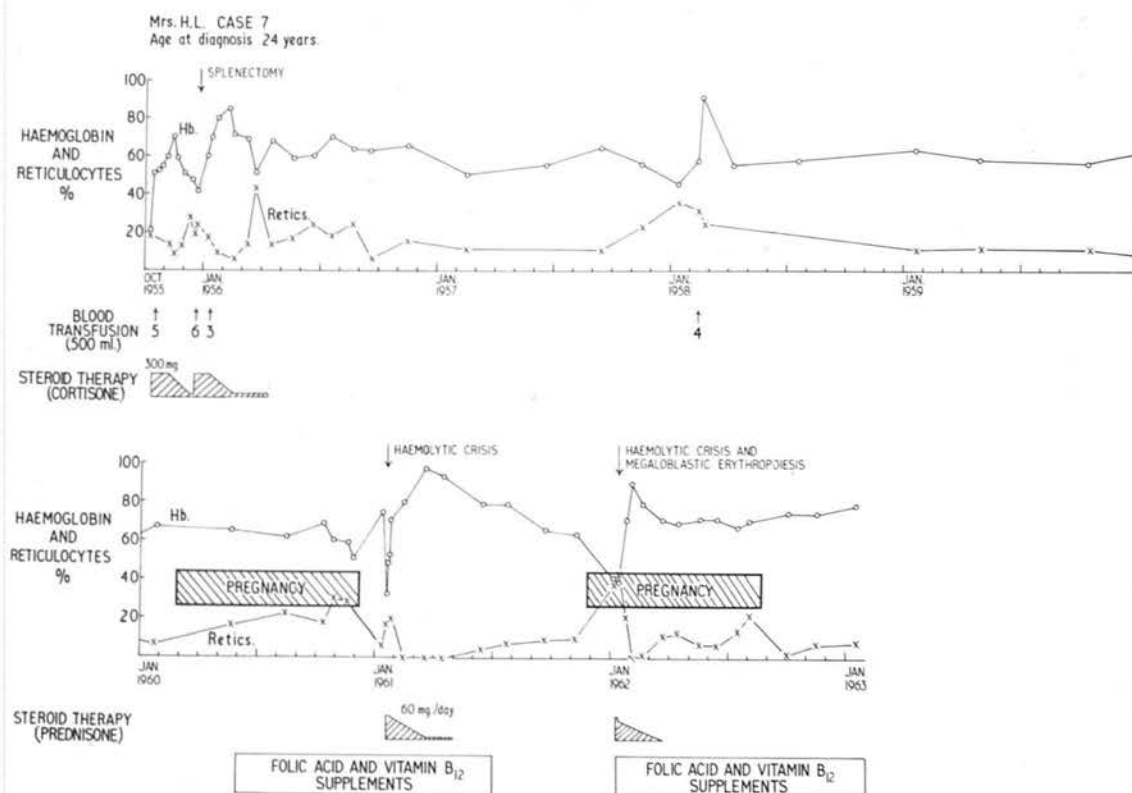


Fig. 19

The course of a patient with chronic relapsing autoimmune haemolytic anaemia. The points that are illustrated are the long periods of compensated haemolysis without recourse to steroid therapy, two haemolytic crises, one while receiving folic acid and vitamin B₁₂ supplements, the other associated with folic acid deficiency, the prompt response to steroid therapy in these two crises and the effect of two pregnancies on the disease.

patient (p. 77); the spleen was small, the antibody was of cold type and there had been marked autoagglutination of the patient's red cells in vitro. In fact, transfusion was required in the immediate post-operative period and in the succeeding days the haemoglobin stabilised in the range 60-70%, reticulocytes 10-15%. The high doses of steroids (now prednisone) used at the time of operation were gradually withdrawn to be stopped after four months, and it was clear (Fig. 19) that while both steroid therapy and splenectomy had had a disappointing effect, an equilibrium between the haemolytic process and the bone marrow's compensatory capacity with a moderate degree of anaemia had been achieved.

This clinical picture (Hb. 50-70%, reticulocytes 5-45%) persisted without change for the next four years until 1960. A 2 litre blood transfusion was given in February 1958 in association with dental extraction; this had the usual transitory effect (Fig. 19).

In 1960 the patient started in her first pregnancy. Antenatal progress was entirely uneventful. During pregnancy the same degree of compensated haemolysis persisted. The usual supplements of iron and vitamin supplements were given, and at the fifth month of gestation, folic acid 5 mg. orally, three times daily, and vitamin B₁₂ injections 250 µg. once per month were added empirically. The patient was confined at full term and gave birth to a male infant who showed no clinical or haematological abnormality; in particular/

particular there was no anaemia, reticulocytosis, abnormal bilirubinaemia or abnormality of the Coombs' antiglobulin reaction.

The early puerperium was uneventful but then after two months, while still receiving vitamin B₁₂ and folic acid, the patient had a severe haemolytic crisis, the haemoglobin level falling to 34%. The only possible reason for this development was infection; members of the patient's family and of neighbouring families had recently suffered from virus hepatitis. There was, however, no evidence of hepatic parenchymal disease in the patient.

Reference has already been made to the dramatic and prompt response to prednisone (Figs. 15 and 19); this remission was much more than anything that had been seen prior to, or at the time of, splenectomy. After the initial good effect prednisone was gradually withdrawn over two months and there was a gradual return of anaemia and reticulocytosis to the levels of the previous four years. Folic acid and vitamin B₁₂ therapy were discontinued in June 1961. Later in the same year (November 1961) the patient started a second pregnancy and towards the end of the second month after the last menstrual period, i.e. after only six weeks' gestation, a second haemolytic crisis supervened (the haemoglobin level falling to 38%). Definite megaloblastic erythropoiesis was now observed in the bone marrow, presumably due to conditioned folic acid deficiency; the level of/

of folic acid in the patient's serum was 4.45 $\mu\text{g./ml.}$ (normal range 4.5 - 15.0 $\mu\text{g./ml.}$). It is noteworthy in this regard that when folic acid deficiency occurs in normal pregnancy, it is not usually significant till the third trimester, and this patient had received vitamin B₁₂ and folic acid supplements as recently as seven months earlier. Moreover, the reticulocytopenia emphasised by Chanarin, Dacie and Mollin (1959) in their patients with megaloblastic erythropoiesis was not seen in this patient; the reticulocyte count was 32-43% during this time.

A prompt remission was again obtained on prednisone therapy, 60 mg. being used in the first instance. This treatment was gradually reduced and stopped over a two month period; pregnancy thereafter proceeded uneventfully and again the patient was delivered of a normal infant (August 1962) at full term. Since then the patient has remained in moderately good health with return to the now familiar picture of compensated haemolysis (Fig. 19).

Comment on Case 7.

A number of interesting points are implied by the management of the foregoing patient.

(i) It has not been necessary to maintain continuous steroid therapy. The patient is not disabled by her average degree of anaemia and prednisone has had a quick and satisfactory effect in the control of crises since splenectomy.

(ii)/

(ii) The two crises observed must have been of different aetiology. It has been suggested that some of the crises seen in haemolytic disease might be due to marrow failure consequent on relative folic acid deficiency; this has for example been recognised as a rare complication of thalassaemia (Chanarin, Dacie and Mollin 1959). Folic acid deficiency cannot, however, have been the mechanism for the first episode since the patient was taking folic acid supplements at the time. However, folic acid deficiency was a very likely mechanism for the second since, not only was the patient established in pregnancy (although at a very early stage), but also she showed a subnormal serum folic acid level and megaloblastic erythropoiesis.

(iii) The patient has successfully been through two normal pregnancies, giving birth without difficulty to two normal children.

(iv) Although high doses of prednisone were required in the first trimester of the second pregnancy and were used with some apprehension, this treatment clearly had no effect on the foetus. This accords with the experience of Frumin, Smith, Taylor and Dratman (1953) who describe the only other reported cases of steroid therapy for autoimmune haemolytic anaemia during pregnancy; their patient received ACTH and cortisone from the second week of pregnancy until term with good haematological remission and without any untoward effects.

3. 12. Fulminating autoimmune haemolytic anaemia; its development in a patient who had previously had a splenectomy for idiopathic thrombocytopenic purpura, its suppression with thymic radiation and subsequent control with a combination of Actinomycin-C and BW 57-322 ("Imuran").

Fulminating haemolytic anaemia leading within weeks or months to a fatal termination is described in most accounts of large series of patients with autoimmune haemolytic anaemia. It was certainly known to Dameshek and Schwartz (1940) who suggested a classification of haemolytic anaemia, which included division of the acquired group into chronic, sub-acute, acute and acute fulminating, on the basis of the course of the patient's illness.

Cases 4, 12 and 15 of the present series showed this type of relentless disease for which no therapy appeared to have anything to offer.

Case 3 presented the same such problem but was unusual in that the violent haemolytic process developed after relapse of idiopathic thrombocytopenic purpura previously successfully treated by splenectomy. Eventually her haemolytic disorder and thrombocytopenia were satisfactorily controlled by the combined use of new antineoplastic drugs (Actinomycin-C and Imuran), and for these reasons, the patient is described in detail.

The patient, an unmarried woman of thirty-five years, was admitted to hospital on 18.10.62 for the second time. She had been known since December 1955 when she was first seen/

seen with idiopathic thrombocytopenic purpura (the diagnosis being based on megakaryocytic hyperplasia in the bone marrow which was otherwise normal and on the absence of splenomegaly). The patient showed no response to prednisone therapy and splenectomy was undertaken in January 1956. There was a satisfactory rise in the platelet count in the post operative period and this remission was sustained during out-patient follow-up, the last regular visit being on 15.10.58, at which time the Hb. level was 89%, platelets 380,000 per c.mm.

When the patient reappeared on 18.10.62 she stated that for some months she had complained of aches and pains in the shoulder girdle; for ten days prior to her visit she had applied Ung. Methyl. Sal. N.F. on her family doctor's prescription. This, after a few days, produced petechiae in the skin over the shoulders, the first time that purpura had been seen since 1956. However, on closer enquiry it transpired that the menses had been abnormally heavy in the previous four months. The initial blood counts were as follows:- Hb. 86%; W.B.C. 10,300 with normal differential count; platelet count less than 10,000 per c.mm.; E.S.R. 2 mm. in the first hour. Later the blood was examined for LE cells on three occasions with negative results, the marrow was found to be cellular but showed a moderate increase of megakaryocytes, and serological study on 5.11.62./

5.11.62. confirmed that a platelet agglutinin was present. A series of agglutination reactions for Brucella and salmonella, the Paul-Bunnell reaction and routine blood culture were negative.

Treatment with prednisone 60 mg. per day (and with oral supplements of potassium chloride) was started on entry to hospital. The steroid therapy was without effect and after two weeks on this dosage gradual reduction of prednisone was arranged. Repeated full blood examination showed no important change except moderate leucocytosis; an example is the result dated 5.11.62: Hb. 89%, W.B.C. 16,500 (of which 86% were neutrophil polymorphs, 9% lymphocytes, 5% monocytes); platelets < 10,000 per c.mm.; E.S.R. 2 mm. in the first hour.

Because of the possibility that the patient's condition was being perpetuated by infection, it was decided to give a short course of antibiotic therapy; significant infection was excluded in the lungs, the genital and the urinary tract, but the culture of the throat swab grew mixed organisms, sensitive to penicillin and to erythromycin. Since the patient had had a previous skin reaction to the former, a course of erythromycin was started on 9.11.62. This antibiotic treatment is believed to be irrelevant to the main events because on 8.11.62 it is certain that there was slight icterus in the patient's sclerae and she felt vaguely unwell. Over/

Over the next two to three days she became progressively more ill, with increasing pallor, acholuric jaundice and throbbing in the ears.

On 12.11.62, the peripheral blood findings were as follows: Hb. 34%; P.C.V. 14%; W.B.C. 44,000 (neutrophil polymorphs 78%, lymphocytes 19%, monocytes 2%, myelocytes 1%, 2 normoblasts per 100 W.B.C.); reticulocytes 2.6%; platelets < 10,000; E.S.R. 142 mm. in the first hour. The direct Coombs' test which had been negative on 5.11.62 was now strongly positive; the reaction was not neutralised by γ globulin and since the antibody in the patient's serum showed some specificity against red cells containing E-antigen and the eluate from sensitised cells did not, it was concluded that more than one antibody was present. The main feature was that a severe haemolytic crisis had developed, due to auto-antibodies in a patient who was receiving high doses of steroid (30 mg. of prednisone per day at the time) and who had already had a splenectomy.

Erythromycin was discontinued, the prednisone was changed to cortisone in a dose of 300 mg. per day and the patient was transfused frequently, as necessary. This regime was continued until 30.11.62, when cortisone was reduced to 200 mg. per day; in the intervening eighteen days an extremely severe haemolytic process continued and the administration of 16 litres of blood was required to keep the haemoglobin level above 50%. The blood picture on/

on 30.11.62, which was typical of others, was: Hb. 67%; P.C.V. 31%; W.B.C. 12,500 (neutrophils 88%, lymphocytes 9%, monocytes 1%, basophils 2%, 3 normoblasts per 100 W.B.C.); reticulocytes 1.7%; platelets < 10,000 per c.mm.; E.S.R. 54 mm. in the first hour. The large amounts of blood administered and the uncontrolled haemolytic process had induced methaemoglobinaemia, rise of the serum bilibubin level to 9.0 mg. per 100 ml. and the appearance of bile pigment in the urine. There was no evidence that the large doses of steroids which had now been in use for six weeks were having any effect.

Although her primary illness was idiopathic thrombocytopenic purpura and thrombocytopenia was still persisting, the patient's survival was more concerned with arrest of the haemolytic process, and consideration was given to the use of antineoplastic agents in view of the encouraging results obtained in autoimmune haemolytic anaemia with 6-mercaptopurine and thioguanine by Dameshek and Schwartz (1962). The regime which was now employed developed from discussion with, and on the advice of Professor M. F. A. Woodruff; the patient was treated first by irradiation to the area of the thymus gland and later by the administration of Actinomycin-C and Imuran, but before describing the further course of the patient a short explanatory digression on the reasons for these measures is necessary.

With/

With regard to the thymus, it is only in the last two years that an important function has been ascribed to it. Burnet (1962) and Miller, Marshall and White (1962) have formulated the hypothesis on the basis of recent experiment that the cells necessary for various functions concerned with the maintenance of bodily integrity and recognised by their capacity to mediate homograft rejection are descendants of cells, which are differentiated for this function in the thymus. These cells normally begin to pass from the thymus into the circulation at the time of birth and are seeded to the spleen and probably lymph nodes where they multiply and develop full immunological tolerance. In addition to this suggested role, the thymus has also been found to show the characteristic changes of immunological activity in examples of autoimmune disease. The most relevant evidence for this is in the mouse strain NZB/BL which spontaneously develops autoimmune haemolytic anaemia with positive direct Coombs' test; in a large proportion of these mice the thymus shows enlargement of the medulla with germinal centres, incomplete lymph follicles and accumulation of plasma cells and mast cells (Burnet and Holmes 1962). On the basis of these early findings, Burnet and Holmes believe that the thymus is the region in which the process of autoimmune disease is initiated.

Because of thrombocytopenia in Case 3, thymectomy could not be contemplated but it seemed reasonable from the foregoing/

foregoing to try the effect of thymic irradiation.

Imuran (BW 57-322) is one of a group of purines related to 6-mercaptopurine (6MP) in which an imidazole ring replaces the hydrogen of the sulphhydryl group of 6MP. Metabolic studies in mice, dogs and humans (Elion, Callahan, Hitchings and Rundles 1960) have shown that Imuran is extensively split, but not instantaneously or completely, in vivo into 6MP and this may permit a steady slow action which is not possible with the parent compound. In preliminary clinical and haematological studies (Rundles, Laszlo, Itoga, Hobson and Garrison 1961), Imuran was administered to twenty-eight patients with various types of leukaemia and autoimmune disease. While there was little to suggest that the agent was qualitatively different from other related compounds in its antitumour activity, platelet suppression rarely occurred, any anaemia was mild in degree and cytopenias produced were rapidly reversed by reduction of dose or suspension of therapy.

Actinomycin-C is a mixture of three related antibiotics isolated from *Streptomyces chrysomallus*. Experimental evidence indicates (Reich, Franklin, Shatkin and Tatum 1961) that Actinomycin interferes with a portion of RNA synthesis and not DNA synthesis, it has a cytostatic effect on lymphoid tissue and may therefore be an inhibitor of antibody production. When used in combination with Imuran, the incidence of side effects is no greater than when Actinomycin-c/

Actinomycin-C is given alone. Because of the different modes of action of these drugs, they might be expected to produce an additive beneficial effect without additive toxicity. In the only published report which refers to the simultaneous administration of Imuran and Actinomycin-C, Murray, Merrill, Dammin, Dealy, Alexandre and Harrison (1962) (and this deals with renal homotransplantation in eighteen patients and 300 dogs) indicate that the combined use of these drugs (and of Imuran and Azaserine) to induce chemical suppression of immune response produced their most encouraging results. Professor Woodruff (personal communication) agrees with this view.

These recent data were the basis for treating Case 3 with Actinomycin-C and Imuran.

The course of the patient's illness during irradiation of the thymus, during the administration of Actinomycin-C and Imuran and during her remission is illustrated in Figs. 20 & 21 and in Table 10.

At the beginning of treatment (4.12.62) the survival time of fresh donor red cells gave a $T_{1/2}^{Cr}$ of 1.5 days.

Irradiation of the thymus was undertaken by Dr. J. Newall through an anterior mediastinal port, beginning on 4.12.62. A total of 500 rads. was given in divided doses. There were certain immediate effects:-

(i) The temperature became normal from the day irradiation started (except on days of transfusion) for the first/

Miss M.F. 35 yrs. CASE 3

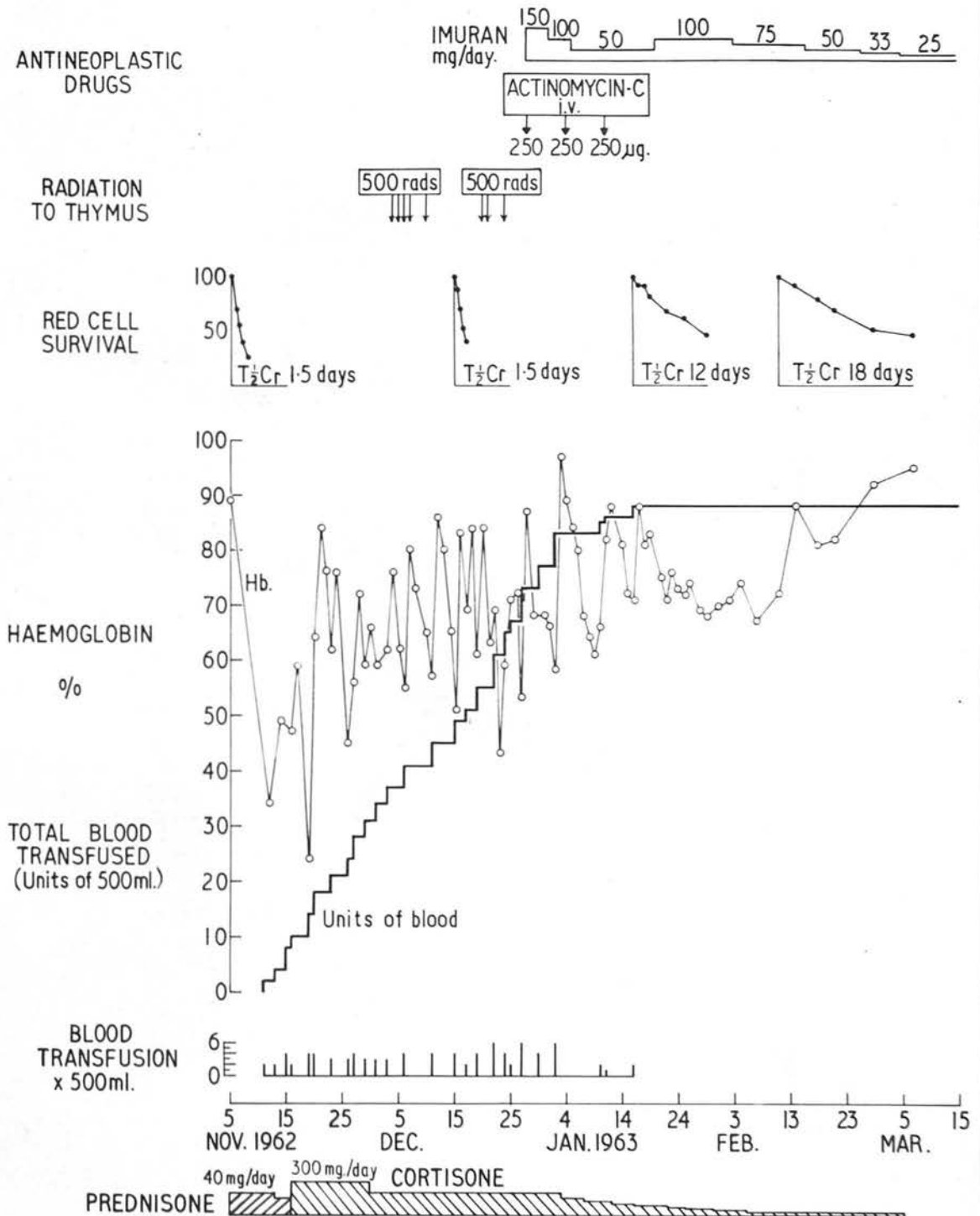


Fig. 20

A patient with fulminating autoimmune haemolytic anaemia which supervened after relapse of thrombocytopenia previously cured by splenectomy. The control of the haemolytic process by the combined use of Actinomycin-C and Imuran is illustrated.

Miss M.F. 35 yrs. CASE 3.

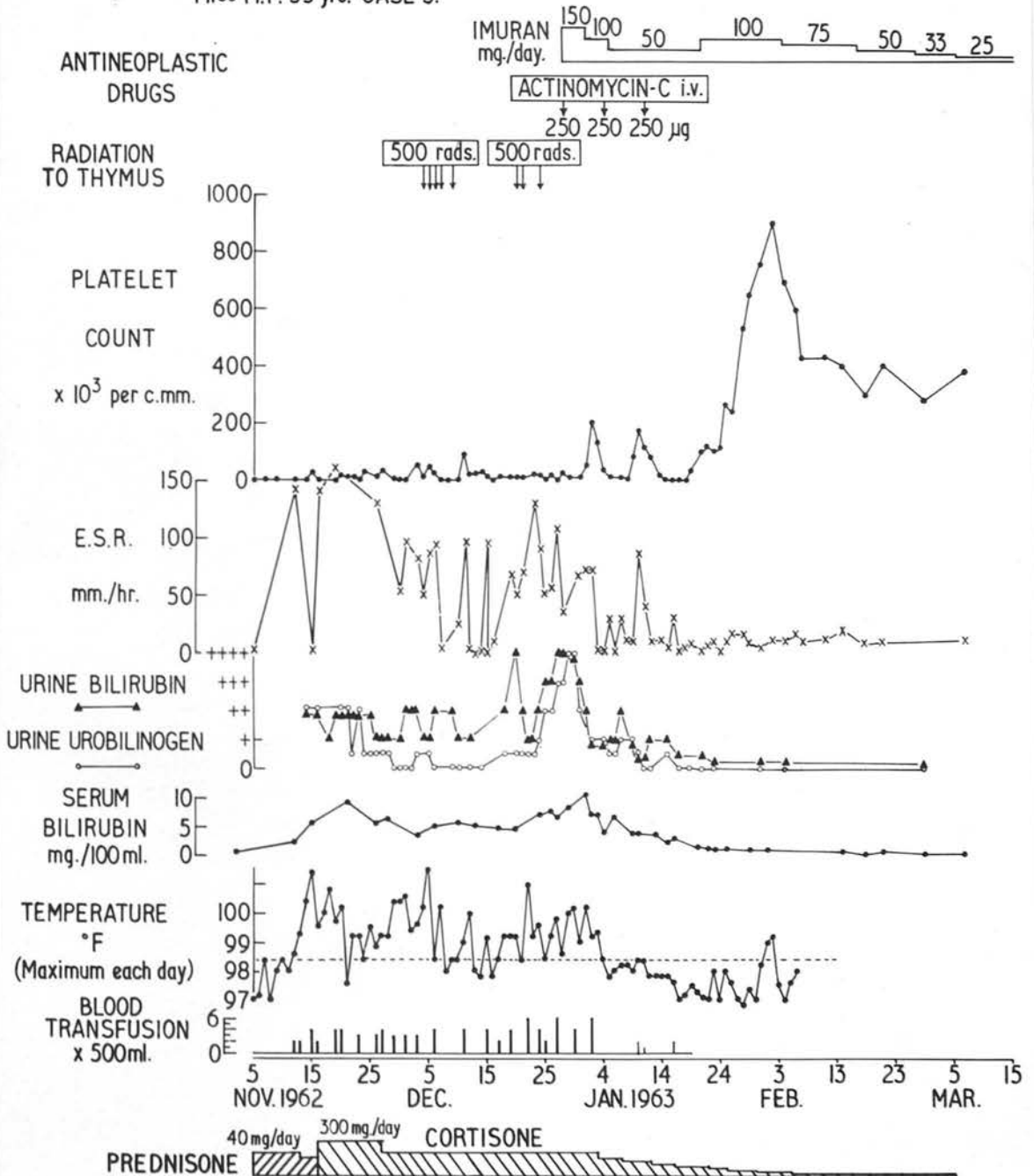


Fig. 21

The effect of thymic irradiation and combined Actinomycin-C and Imuran therapy on thrombocytopenia, E.S.R., bile pigment levels and temperature in Case 3.

TABLE 10

SERIAL DIRECT COOMBS' TEST TITRATIONS IN CASE 3 (M. F.)

Treatment	Date	Dilution of Antiglobulin Serum									
		1/10	1/20	1/40	1/80	1/160	1/320	1/640	1/1280	1/2560	
4.12.62 First course of thymic irradiation	4.12.62	3	3	3	3	2	1	1	(W)	-	
	6.12.62	3	3	2	1	(W)	-	-	-	-	
	11.12.62	3	2	1	-	-	-	-	-	-	
	14.12.62	3	2	2	1	-	-	-	-	-	
	17.12.62	4	3	1	1	(W)	-	-	-	-	
	20.12.62	4	3	3	2	(W)	-	-	-	-	
20.12.62 Second course of thymic irradiation	22.12.62	4	3	3	2	(W)	-	-	-	-	
	24.12.62	4	4	3	2	1	(W)	-	-	-	
28.12.62 Beginning of combined Actinomycin-C and Imuran therapy	29.12.62	4	4	3	2	1	(W)	-	-	-	
	3.1.63	3	2	1	(W)	-	-	-	-	-	
	10.1.63	3	2	(W)	-	-	-	-	-	-	
	14.1.63	4	4	4	3	1	1	(W)	-	-	
	16.1.63	4	4	3	2	1	(W)	-	-	-	
	21.1.63	4	3	2	1	1	(W)	-	-	-	
	24.1.63	4	4	2	1	1	(W)	(W)	-	-	
	28.1.63	3	3	2	1	(W)	-	-	-	-	
	31.1.63	3	3	2	1	1	(W)	(W)	-	-	
	4.2.63	3	2	1	(W)	(W)	-	-	-	-	
	7.2.63	3	2	1	1	(W)	(W)	-	-	-	
	11.2.63	2	1	1	(W)	-	-	-	-	-	
	15.2.63	3	2	1	1	1	-	-	-	-	
	18.2.63	2	2	2	1	(W)	(W)	-	-	-	
	21.2.63	2	2	2	1	(W)	-	-	-	-	

The above figures refer to strength of agglutination
e.g. (3 = +++); (W) = weak agglutination;
- = no agglutination.

first time since the onset of the haemolytic process.

(ii) The transfusion requirement fell from an average of 0.8 litres per day in the twenty days preceding irradiation to an average of 0.5 litres per day in the ten days beginning with the first day of radiotherapy.

(iii) The quantitative Coombs' test titre fell from 1/1280 on 4.12.62 to 1/160 on 6.12.62 to 1/40 on 11.12.62.

(iv) The E.S.R. which had been 130, 54, 96 and 82 mm. in the first hour in the ten days preceding irradiation was 85, 94, 4, 25, 95, 3 and 1 mm. in the first hour in succeeding readings from 4.12.62 to 14.12.62.

(v) The platelet count reached a level of 90,000 per c.mm. on 11.12.62, the highest value recorded since the patient's admission to hospital on 18.10.62.

These indices of improvement were not sustained and from 14.12.62 there was a rapid return to the previous transfusion requirement, and the titre of the Coombs' test gradually increased, the reaction being positive at 1/60 on 17.12.62 and at 1/320 on 22.12.62. A second measurement of survival time of fresh donor cells, starting on 17.12.62 (too late for the maximum remission) gave a $T_{1/2}^{Cr}$ of 1.5 days.

Encouraged by the first response it was decided to use further thymic irradiation; 500 rads. were again given through the same anterior chest port in divided doses between 20.12.62 and 24.12.62. On this occasion there was no response that could be measured; if anything the transfusion/

fusion requirement accelerated, the pyrexia which had returned did not settle, there was no improvement in the platelet count or in the E.S.R. and the titre of the direct Coombs' test remained the same. In addition the serum bilirubin rose to 11.2 mg./100 ml. and methaemalbinaemia and bilirubinuria returned.

On 28.12.62 the following regime was started: Actinomycin-C, 250 µg. by intravenous infusion at weekly intervals (this was given on three occasions), and Imuran 150 mg. daily for four days, reducing to 100 mg. thereafter.

Subsequent events are best seen in Figs. 20 + 21. Immediately the serum bilirubin level and bile pigment excretion began to fall as did the transfusion requirement. (By 2.1.63 42 litres of blood had been given and only one transfusion had produced any reaction, an urticarial rash mentioned on p. 90). In the first three weeks of drug therapy, only 2.5 litres of blood were required and on 16.1.63 a third estimate of red cell survival gave a $T_{1/2}^{Cr}$ of 12 days. The titre of the direct Coombs' test again fell to 1/40 and ten days after starting drug treatment the temperature became normal.

Cortisone which had been maintained at a level of 200 mg. daily till 3.1.62 was now gradually reduced, no further Actinomycin-C was given after 11.1.62 and the dose of Imuran was varied between 50 and 100 mg. as illustrated.

From/

From 16.1.63 no further blood transfusion has been necessary, the haemoglobin level has risen to levels above 80%, the serum bilirubin level has returned to normal and there has been no bile pigment in the urine. This situation has persisted to the time of writing, 28.2.63 (i.e. for six weeks), and all the investigative findings suggest that the haemolytic process has been suppressed. The patient, at the time of writing (28.2.63), is receiving Imuran 50 mg. on alternate days and cortisone 25 mg. per day. The red cell survival measurement started on 11.2.63 gave a $T_{1/2}^{Cr}$ of 18 days.

Frequent marrow sampling has not been undertaken and did not seem to be necessary. The last aspirate (from the ilium) on 17.1.63 showed a hypercellular picture with normoblastic hyperplasia and an excess of megakaryocytes. There is no indication that the drug therapy has induced any leucopenia, regular white cell counts (at the time of writing) being in the range 6,900 to 14,800 per c.mm. with normal differential count.

Apart from the dramatic remission in the haemolytic process the thrombocytopenia has also been corrected by drug therapy.

In the early stages there were transient increases in the platelet count into the normal range, a peak following each infusion of Actinomycin-C. However, beginning on 19.1.63 (after the third and last infusion of Actinomycin-C)

a sustained remission of thrombocytopenia was noted. The platelet count reached a level of 900,000 on 2.2.63 and has since then (till 28.2.63) been maintained in the region of 400,000.

Apart from some fluid retention during the period of cortisone treatment and the isolated transfusion reaction referred to above, there were no side effects from any of the therapeutic measures and the patient considers that she has been returned to normal health (28.2.63.).

Comment on Case 3.

(i) Considerable interest attaches to the simultaneous appearance of autoimmune haemolytic anaemia and thrombocytopenia. Evans and Duane (1949) and Evans, Takahashi, Duane, Payne and Liu (1951) drew attention to the frequent association and postulated that when leucopenia and thrombocytopenia co-existed with a haemolytic process, auto-antibodies might also be implicated in the suppression of white cells and platelets. Several authors, notably Crosby (1955), Crosby and Rappaport (1957), Dacie (1954), Chertkow and Dacie (1956) and Dacie (1962), have emphasised the sinister prognosis which the presence of thrombocytopenic purpura with haemolytic anaemia carries. The precise incidence of the association is not known but it reached 13.2% in the series of 83 cases of idiopathic disease of warm antibody type in the series of Dausset and Colombani (1959). The sequence of events varies in that haemolytic disease/

disease may precede the appearance of thrombocytopenia or vice versa, or they may appear simultaneously. The most unusual patients are those in whom a fulminating haemolytic process has followed at an interval after splenectomy for idiopathic thrombocytopenic purpura; an example of this rare occurrence was described by Waugh (1932) and Dacie (1962) refers to two others in his own experience which were described in his earlier papers (Dacie and De Gruchy 1951, Dacie 1954). The foregoing case appears to be the only additional example in the literature.

(ii) The fulminating nature of the haemolytic process is illustrated in this patient by the very large transfusion requirement. Forty-two litres of blood were given in six weeks with only one minor reaction.

(iii) The autoimmune haemolytic anaemia supervened while under observation and while the patient was receiving large doses of prednisone.

(iv) Significant but transitory improvement in transfusion requirement, Coombs' test titre, temperature, E.S.R. and platelet count succeeded irradiation of the thymic region but could not be repeated by a second course of radiotherapy.

(v) The patient's successful management confirms the possibility of arresting autoimmune haemolytic disease, which is otherwise uncontrollable by steroid therapy and splenectomy, using antineoplastic drugs. This is believed to be the first patient in which Actinomycin-C and Imuran have/

have been employed for this purpose.

(vi) Since idiopathic thrombocytopenic purpura may be caused by an autoimmune reaction, the experience in this case suggests that antineoplastic drugs may have a place in the management of this condition also, when other measures have failed.

3. 13. Main conclusions from the studies on patients with autoimmune haemolytic anaemia.

(i) No particular clinical features have marked the group of 23 patients with autoimmune haemolytic anaemia that has been discussed. The mode of presentation has varied, being usually due to acute (the illness being judged to begin with a haemolytic crisis in 6 patients) or chronic anaemia. Acholuric jaundice was definitely present in only 15 of the patients initially; the serum bilirubin level was within the normal range in 5 patients and there was no urobilinogenuria in the early stages of examination in another 4, despite evidence of a moderately severe haemolytic process.

Gross splenic enlargement was encountered only once and this was in a patient with lymphosarcoma. In 7 of the 16 patients with idiopathic disease the spleen was actually impalpable at the time of diagnosis.

The most useful indicators of a possible autoimmune haemolytic process in the early stages of investigation are considered to be anaemia, unexplained reticulocytosis (present/

(present in all but two patients) and a raised E.S.R. With regard to the last, the mean value for the patients with idiopathic disease was 82 mm. in the first hour; in only three instances was it less than 45 mm. when first estimated.

(ii) Megaloblastic erythropoiesis was encountered on four occasions; in one patient this developed during the course of her illness, in the other three it was present initially. This finding appeared to have different causes. In two subjects (one of whom had previously had a gastro-enterostomy, and the other was over 70 years of age), the serum vitamin B₁₂ level was subnormal; in the third, the change in the marrow was observed after a haemolytic crisis in the initial stages of pregnancy, at a time when the serum folic acid level was subnormal; in the fourth patient the serum vitamin B₁₂ level was well within the normal range and the development was presumed to have been due to conditioned folic acid deficiency.

(iii) There were no significant changes in the plasma protein pattern, except those in the symptomatic group which could be ascribed to the underlying disease; there was no indication of preponderance of a particular blood group in this series of patients; the experience of the nature of the red cell antibody and the incidence of warm and cold types corresponded with that of others.

(iv) While the course of illness in the patients in the symptomatic/

symptomatic^{group} is mainly dependent on the underlying disease, the natural history of the autoimmune haemolytic process in the series of patients studied has followed distinct paths:

- (a) Self limiting disease with conversion of the direct Coombs' test to a negative reaction, 3 patients;
- (b) Sustained remission for weeks, months or years with complete suppression of signs of haemolysis or well compensated haemolysis, 7 patients;
- (c) Chronic relapsing course, 5 patients (2 of these have the cold haemagglutinin syndrome);
- (d) Uncontrolled relentless haemolytic disease, 8 patients (all but one of these patients have died).

(v) Transfusions have been employed frequently because of recurring severe anaemia and in four patients the giving of blood at intervals was necessary for the patient's survival. Despite the difficulty of cross matching blood due to the presence of red cell antibodies and the frequency with which these patients develop iso-antibodies, only minor transfusion reactions have been encountered; one patient with a fulminating haemolytic process required 42 litres of blood in the course of six weeks. The impression has been gained that the giving of a large transfusion in the initial stages of illness may temporarily subdue the haemolytic process and this might gain time until steroid therapy became effective; possible reasons for this effect of transfusion have been advanced.

(vi) All of the 23 patients received steroid therapy at some stage of their illness. This was found to have no benefit in 11, very transitory benefit in 4, but has formed the main line of treatment, producing sustained remission on acceptable maintenance doses or on withdrawal of treatment, in the remaining eight. Attention is drawn to the high incidence of serious side effects; 7 patients had one or more important complications, which included significant fluid retention (in 3), perforation of a viscus (in 2) and burst abdomen after splenectomy (in 2).

(vii) Nine patients in the series were submitted to splenectomy; this can be judged to have produced partial remission in only three and a good result (but not clinical cure) in only one.

In 12 of the 18 patients in whom red cell survival measurements were made with chromium-51, data were also collected using body surface measurements of radioactivity to evaluate the sequestration of red cells by the spleen in the haemolytic process. In the five patients for whom these results are available, who proceeded to splenectomy, the spleen sequestration index alone or the spleen/liver ratio at $T_{1/2}Cr$ alone (p. 48) does not adequately describe the role of the spleen. The first does not take into account the uptake of radioactivity by the liver and the second excludes the effect of a high blood flow through the spleen/

acid and might have been precipitated by infection, the second occurred early in the second pregnancy and was almost certainly due to folic acid deficiency. Both of these crises were rapidly responsive to steroid therapy; the initial crisis prior to splenectomy was only partially influenced by cortisone.

Large doses of prednisone were given in the first trimester of the second pregnancy without any adverse effect on the mother or foetus.

(c) Case 3 developed a fulminating autoimmune haemolytic anaemia after relapse of thrombocytopenia, previously successfully treated by splenectomy six years earlier. No remission was evident following large doses of adrenocortico-steroids and massive blood transfusion was required. The disease was definitely but transiently influenced by irradiation of the thymus; remission was finally secured by the combined use of Actinomycin-C and Imuran. This is believed to be the first account of control of an autoimmune haemolytic process using these particular drugs and the first account of correction of antibody-induced thrombocytopenia using any form of anti-neoplastic agent.

CHAPTER FOUR

SYMPTOMATIC NON-IMMUNE HAEMOLYTIC ANAEMIA4. 1. Introduction.

Dameshek and Schwartz (1940) are believed to have introduced the term "symptomatic", later popularised by Singer and Dameshek (1941), to designate those cases where an aetiological relationship appeared to exist between acquired haemolytic anaemia and an underlying disease process. These investigators were aware that the role of haemolysis as an important cause of anaemia in various conditions had been widely accepted; the first accounts of the occurrence in leukaemia are attributed to Hirschfeld (1906) and Pappenheim (1906), and after these reports a haemolytic syndrome was noted, particularly in other cases of leukaemia (Brill 1924, Watson 1939), in reticuloses (Davidson 1932), carcinomatosis (Waugh 1936) and liver disease (Lovibond 1935); sarcoidosis and tuberculosis had also been implicated. Attention was drawn to the frequency and significance of this haemolytic anaemia in the diseases cited by the further accounts of Davis (1944) and of Stats, Rosenthal and Wasserman (1947).

These earlier descriptions dealt with clinically evident haemolytic anaemia, usually with reticulocytosis and acholuric jaundice, and emphasis was often placed on the presence of macrocytosis in the peripheral blood (Davidson 1932/

spleen relative to the liver. These criticisms are met by using a multiple of the spleen sequestration index and the spleen/liver ratio which has been termed the "Spleen Number".

The only remissions from splenectomy were seen when the Spleen Number exceeded 300. The same treatment of data for patients who were managed with steroids alone showed that the two patients with the highest Spleen Numbers had had the most satisfactory remissions from steroid therapy.

(viii) Three exceptional patients have been encountered and have been described in detail.

(a) Case 1 suffered from a condition termed "familial haemophagocytic reticulosis". Two of his three siblings had already died of the disease and evidence of abnormalities were obtained in the father (positive Coombs' test on one occasion; reduced red cell survival). The outstanding feature of the condition was very marked histiocytic proliferation with erythrophagocytosis.

(b) Case 7 has been followed for seven years with a chronic relapsing haemolytic process. She has been observed through two pregnancies with partially compensated haemolysis. She has had two haemolytic crises, apparently of different aetiology; the first occurred while receiving supplements of vitamin B₁₂ and folic acid/

1932, Lovibond 1935). The extended use of red cell survival measurements subsequently demonstrated, however, that decreased erythrocyte life span was present relatively frequently in leukaemias and reticuloses and disorders of connective tissue, whether or not the patient gave evidence of overt haemolysis, and also in an additional number of conditions not previously regarded as showing any haemolytic component; the latter included inflammatory diseases (e.g. rheumatoid arthritis, Freireich, Ross, Bayles, Emerson and Finch 1954; Alexander, Richmond, Roy and Duthie 1956), chronic renal disease (Emerson and Burrows 1949, Chaplin and Mollison 1953) and miscellaneous anaemias (Brown, Hayward, Powell and Witts 1944 and Brown 1950).

The subject was extensively reviewed by Wasserman, Stats, Schwartz and Fudenberg (1955) who emphasised that, while reduction of red cell life span was the central feature in a miscellany of symptomatic anaemias, there seemed to be two distinct groups both clinically and haematologically. There were those patients on the one hand who usually presented classical signs of haemolysis with bilirubinaemia, reticulocytosis and marrow hyperplasia in whom the haemolytic process was clearly the main cause of anaemia. On the other hand there was an ill-defined group in whom overt signs of haemolysis were not conspicuous and the main features were those of the primary disease; the recognition of a haemolytic process in them was based solely on red cell survival/

survival measurement, the marrow was not hyperactive and increased blood destruction appeared to be only a contributory factor in the production of a subnormal haemoglobin level. Moreover, Wasserman and his colleagues observed that in the first group there was frequently a positive Coombs' test, the form of red cell destruction tended to be an exponential function and there was often a good effect from steroid therapy; in the second group there was no evidence for an auto-immune mechanism, although the shortened red cell life span was due to extracorporeal factors, red cell destruction was a linear process and only improvement in the underlying disease had any influence on the anaemia.

Studies in auto-immune haemolytic anaemia have been described in the previous chapter; the "symptomatic" group accounted for approximately one third of the total series of patients, and it is re-emphasised that the development of a red cell antibody is only common when the primary disease is a lymphoproliferative condition or disseminated lupus erythematosus. This chapter deals with a selected group of patients in whom excessive blood destruction could not be explained by an auto-immune process.

Examples of the writer's findings of reduced red cell survival in carcinomatosis, uraemia, hepatic cirrhosis and Addisonian pernicious anaemia were illustrated in Fig. 6

(p. 53); collaborative studies of the haemolytic element in/

in the anaemia of rheumatoid arthritis have been reported in the literature (Alexander, Richmond, Roy and Duthie 1956, Richmond, Alexander, Potter and Duthie 1961). Particular experience has been obtained, in addition, of red cell survival measurements in cases of leukaemia, reticulosis and primary myeloid metaplasia. These last patients were studied either because they had some indicators of overt haemolysis (bilirubinaemia, reticulocytosis), or because they had a large transfusion requirement, or because they had significant anaemia which could not be adequately explained. Twenty-seven patients with these diseases were found to have adequate data and because of the relationship between the primary diseases and the similar problems of management which they create it has been felt justifiable to restrict the considerations in this chapter to this group; the basis for inclusion has been reduction of red life span below the normal range ($T_{1/2}Cr$ less than 24 days) and negative direct Coombs' test. In the absence of any pathogenetic mechanism implicating red cell antibodies, the haemolytic process has been termed "symptomatic non-immune haemolytic anaemia" since this, in the present state of knowledge, most adequately describes the condition. The haemolytic process will be shown to vary from mild in degree to severe; anaemia may be considerable and disabling and possible therapeutic measures are discussed.

4. 2. Main clinical features of the group of twenty-seven patients with symptomatic non-immune haemolytic anaemia and reasons for study.

The details considered in this section are summarised in Table II. Case 24 was observed over a six year period. In the first stages of illness a haemolytic process was associated with myelofibrosis; terminally re-investigation was required because of the return of symptomatic haemolytic anaemia due to transformation of the primary condition to acute leukaemia. The observations in the latter period were distinct from the first and will be described under Case 24b.

Since the group of patients was selected no importance is attached to age and sex distribution; there were 15 female patients and 12 males, the age range was 22 years to 89 years, mean 53.8 years.

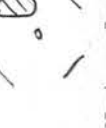
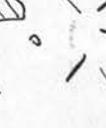




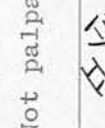

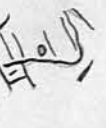
The numbers of patients suffering from each of the diseases studied were:-

Acute leukaemia (following post-tuberculous myelofibrosis)	1
Chronic myeloid leukaemia	3
Chronic lymphatic leukaemia	6
Hodgkin's disease	8
Lymphosarcoma	4
Myeloid metaplasia with and without myelofibrosis	5

The known duration of primary disease varied from two months/

TABLE II

THE MAIN CLINICAL AND HAEMATOLOGICAL FEATURES IN 27 PATIENTS WITH A SYMPTOMATIC NON-IMMUNE HAEMOLYTIC PROCESS ACCOMPANYING LEUKAEMIA, RETICULOSIS OR MYELOID METAPLASIA

Patient	Age in Years	Sex	Primary Disease	Possible Duration of Primary Disease	Clinical and Haematological Findings Prior to Red Cell Survival Measurement					T _{1/2} Cr in days
					Hb. %	Retics. %	Serum Bilirubin mg./100 ml.	Spleen Size (approximate)	Bone Marrow	
24a.	26		Post tuberculous myelofibrosis	9 months	34	< 1	0.4		Myelofibrosis	9
24b.	31	F	Acute myelogenous leukaemia	5 years	48	< 1	0.5		Acute myelogenous leukaemia	13
25.	30	F	Hodgkin's disease	7 years	67	< 1	-	Not palpable	Increase in small lymphocytes	21
26.	65	M	Chronic lymphatic leukaemia	3 years	37	1.5	0.5		Lymphocytic infiltration	21
27.	29	F	Hodgkin's disease	5 years	85	< 1	1.6		Normoblastic hyperplasia	14
28.	59	M	Chronic lymphatic leukaemia	10 years	54	< 1	1.2		Lymphocytic infiltration	20
29.	68	F	Chronic lymphatic leukaemia	13 years	76	< 1	0.2	Not palpable	Lymphocytic infiltration	23
30.	64	M	Chronic myeloid leukaemia	2 months	49	3.0	0.5		Increase in myeloid series	13
31.	22	M	Hodgkin's disease	2 years	78	< 1	-		-	22
32.	48	F	Hodgkin's disease	5 years	72	2	-	Not palpable	-	18
33.	50	M	Myelofibrosis	3 years	45	2.1	0.1		Myelofibrosis	13
34.	61	F	Chronic lymphatic leukaemia	4 years	50	4	-		Lymphocytic infiltration	16
35.	49	F	Lymphosarcoma	5 years	67	2	0.7		Normoblastic hyperplasia	9
36.	83	M	Chronic myeloid leukaemia	2 years	62	< 1	0.8		-	1.5
37.	47	M	Hodgkin's disease	1 year	56	< 1	1.0		Hypocellular	22
38.	58	M	Chronic lymphatic leukaemia	10 years	56	1.3	0.3		Lymphocytic infiltration	22
39.	55	F	Lymphosarcoma	2 years	77	1.1	-		Normoblastic hyperplasia	23
40.	56	F	Primary myeloid metaplasia	4 years	67	< 1	0.7		Normal	12
41.	89	M	Chronic lymphatic leukaemia	18 months	61	< 1	0.3		Lymphocytic infiltration	22
42.	59	F	Chronic myeloid leukaemia	3 months	51	2.0	0.3		Increase in myeloid series	15
43.	67	F	Lymphosarcoma	7 years	77	2.0	0.5		-	18
44.	48	F	Hodgkin's disease	18 months	47	4.8	2.3	Not palpable	-	3
45.	73	F	Myelofibrosis	12 years	43	-	1.5		Myelofibrosis	13
46.	56	M	Myelofibrosis	5 years	60	3.3	0.6		Myelofibrosis	20
47.	28	F	Hodgkin's disease	3 years	81	0.2	0.3		-	13
48.	67	M	Hodgkin's disease	4 years	67	< 1	0.4	Not palpable	Normoblastic hyperplasia	18
49.	64	M	Lymphosarcoma	2 years	54	2	1.0		Increase in lymphocytes	11
50.	55	F	Myelofibrosis	8 years	21	8	0.9		Myelofibrosis	16

months to twelve years, but in twenty-two patients the primary condition had been present for two years or more before the clinical picture led to suspicion of a symptomatic haemolytic process.

It will be seen from Table II that the degree of splenic enlargement was very variable. In five patients (Cases 25, 29, 32, 44 and 48) the spleen was impalpable at the time of investigation but was moderately to considerably enlarged in eighteen of the others. Only one patient (Case 44) was clinically jaundiced.

The reasons for consideration of haemolytic disease in individual patients fell into four categories:-

(i) Signs suggesting overthaemolysis; reticulocytosis or bilirubinaemia or both. Four patients (Cases 30, 44, 46 and 50). (It should be stated that reticulocytosis need not have any significance in patients with myeloid metaplasia since, in this condition, primitive white cells and red cells are regularly found in the circulation).

(ii) Large transfusion requirement. Eight patients (Cases 24, 26, 28, 33, 36, 40, 42 and 45).

(iii) Moderate to severe anaemia. Twelve patients (Cases 25, 31, 32, 34, 35, 37, 38, 39, 41, 43, 48 and 49).

(iv) History of recurring anaemia in recent months. Three patients (Cases 27, 29 and 47).

4. 3. Red cell survival time in relation to clinical and routine investigative findings.

One of the problems in evaluating the significance of the haemolytic process in symptomatic non-immune haemolytic disease is the multiplicity of factors that are also affecting erythropoiesis. The obvious confusions arise from invasion of the marrow by foreign cells, the unknown action of splenomegaly and the residua of treatment, notably with alkylating agents and radiotherapy, on the bone marrow. In addition, the common occurrence of thrombocytopenia makes it essential to exclude occult bleeding from the alimentary tract.

The $T_{1/2}Cr$ which has been taken as the only criterion for excessive blood destruction in this series of patients ranged from 1.5 days to 23 days; the mean value was 15.8 days. When the $T_{1/2}Cr$ is 20 days or more, haemolysis cannot be seen as having more than a minor role in the genesis of anaemia in these patients with leukaemia, reticulosis and myeloid metaplasia. Such a value was obtained in ten subjects; five of these (Cases 25, 26, 28, 29 and 38) had lymphocytic infiltration of the bone marrow, one (Case 46) had myelofibrosis and one (Case 37) had a hypocellular marrow which was possibly due to previous treatment with nitrogen mustard. It will be noted that two of these patients with $T_{1/2}Cr$ exceeding 20 days (Cases 26 and 28) were investigated primarily because of their need for frequent transfusion./

transfusion.

Below this arbitrary value for $T_{1/2}^{Cr}$ of 20 days, it seems reasonable to accept the red cell survival time as having increasing importance in determining the patient's haemoglobin level, and below 15 days (ten patients) it is probably the major factor.

The haemoglobin level in the series varied from 21% to 85% (mean 58.6%) and in seven patients it was below 50% at the time of investigation. The reticulocyte count exceeded 2% in only six patient but two of these had myelofibrosis in which condition the appearance of primitive white cells and red cells in the circulation has already been mentioned; that too much importance should not be attached to reticulocytosis in myeloid metaplasia is illustrated by Case 46 who had a reticulocyte count of 3.3%, but in whom the $T_{1/2}^{Cr}$ was 20 days. Conversely, it is notable that the most marked reduction of $T_{1/2}^{Cr}$ (Case 36, $T_{1/2}^{Cr}$ 1.5 days), observed in a patient with chronic myeloid leukaemia, was associated with no reticulocyte response.

The serum bilirubin level exceeded 1.0 mg. per 100 ml. in only four patients. This measurement is also difficult to evaluate in a group of subjects in whom liver infiltration by the primary disease occurs commonly; in only one of these patients (Case 44) were routine tests of liver function entirely normal. In Case 44 there was acholuric jaundice; this patient also had reticulocytosis and is the only one in the/

the whole series of twenty-seven patients in whom it can be stated that overt signs of haemolytic disease were present.

Study of the data in Table I suggests that there is no correlation between spleen size and the haemolytic process in this series; the extreme examples are Cases 28 and 44. In the former the spleen was greatly enlarged and the $T_{1/2}Cr$ 20 days, in the latter the spleen was impalpable and the $T_{1/2}Cr$ 3 days.

Examination of the clinical and routine investigative data relating to these twenty-seven patients confirms the view that in a symptomatic non-immune haemolytic process, signs of haemolysis (increased pigment turnover and compensatory activity on the part of the marrow) are rarely present even when the red cell life span is markedly shortened. In this context increased red cell breakdown is only demonstrable from measurement of red cell survival.

4. 4. Mechanisms of haemolysis in symptomatic non-immune haemolytic anaemia.

In their consideration of symptomatic haemolytic anaemia not due to circulating red cell antibodies, Wasserman, Stats, Schwartz and Fudenberg (1955) made much of the linear form of the red cell survival curve in their patients compared with the exponential curve indicating random destruction of red cells seen in the autoimmune disease. The linear removal of cells differed only in slope from that seen in normal subjects, and since the removal of normal/

normal cells from the blood is believed to be the consequence mainly, if not solely, of ageing, they argued that these red cell survival data suggested accelerated ageing of the cells as the mechanism of increased red cell destruction in symptomatic non-immune haemolytic anaemia.

Although no experimental evidence has been obtained in support of this view, Dameshek and Schwartz (1959) also regard this explanation as the likely effect of "the sick circulation" (haemopathic haemolysis).

In this regard Crosby and Benjamin (1957) have been successful in demonstrating that abnormal haemolysis occurs in vitro in the blood of patients with leukaemia and malignant disease during sterile incubation for twenty-four hours; the effect is not seen until after a latent period of six to eight hours; haemolysis is inhibited by excess glucose, by a low pH and by removal of calcium from the plasma. Later Crosby, Vullo and Garriga (1961) found, however, that this haemolytic system in the patient's plasma cannot be correlated with shortened red cell survival.

There seem to be no other experimental data indicating that an extracorporeal factor may alter red cell integrity in symptomatic non-immune haemolytic anaemia.

The relationship of the haemolytic tendency to splenomegaly has been stressed (Berlin 1951) but this was not constant in the experience of Desforges, Ross and Moloney (1960) and is not evident from the features of the present series/

series of patients. Nevertheless, in many reports dealing with the subject, splenectomy has been found to be beneficial in occasional patients; this was the experience, for example, in a series of patients with leukaemia and leukaemia sarcoma (Fisher, Welch and Dameshek 1952), various malignant diseases, (Stats, Rosenthal and Wasserman 1947), myelogenous leukaemia (Jonsson, Hansen-Pruss and Rundles 1950) and Hodgkin's disease (Sykes, Karnofsky, McNeer and Craver 1954). There is also evidence that in myeloid metaplasia, despite the role of the spleen in extramedullary haemopoiesis in this condition, splenectomy may sometimes successfully reduce a haemolytic process (Loeb, Moore and Dubach 1953, Green, Conley, Ashburn and Peters 1953, Cartwright, Finch, Loeb, Moore, Singer and Dameshek 1955, Richmond and Duncan 1956). The very variable and unpredictable results of splenectomy are, however, illustrated in the data of Wasserman and his colleagues who discuss twenty-nine patients with symptomatic haemolytic anaemia; splenectomy or irradiation of the spleen was undertaken in sixteen patients. Nine of these were in the "non-immune" group, two obtained a "good" result and two a "fair" result; in the remainder no remission was found. Of the remaining seven with autoimmune haemolytic anaemia only two showed benefit from either form of treatment.

4. 5. Basic therapeutic measures.

The data discussed in this section are summarised in Table 12.

With the exception of the six patients who were suffering from myeloid metaplasia (Cases 24, 33, 40, 45, 46 and 50) and one with chronic myeloid leukaemia (Case 30), each individual had received or was receiving chemotherapy or radiotherapy (other than to the spleen) for his primary disease at the time shortened red cell survival was demonstrated. The patients studied in America were more readily treated with chemotherapeutic agents and received a wider variety of these drugs than did the patients investigated in Edinburgh; this was, however, due to the fact that the Memorial Hospital, New York, was a centre for the clinical trial of antineoplastic preparations. Local irradiation was widely applied to the patients with reticulosis and chronic lymphatic leukaemia for the treatment of local accumulation of disease (e.g. in gland masses, lungs, retroperitoneal space).

Eighteen patients required blood transfusions and in eight of these (p. 137) the requirement was large and frequent because of recurring severe anaemia. Seventeen patients (Table 12) were given steroid therapy, either as part of the management of their primary disease or in an attempt to correct their anaemia. While it was impossible to form definite conclusions as to the effect of this when several/

TABLE 12

BASIC FORMS OF TREATMENT IN 27 PATIENTS WITH SYMPTOMATIC NON-IMMUNE

HAEMOLYTIC ANAEMIA

	24a	24b	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50
	EB	EB	BC	JC	TD	EF	EG	JH	MG	AH	WH	EH	CM	SM	HM	WQ	MR	JR	PR	MR	AR	AS	RS	NS	US	FT	GV	AW
patient	-	+	+	+	-	+	+	-	-	-	-	-	+	+	+	+	+	-	+	+	-	+	-	-	+	-	+	-
chemotherapy	-	-	+	+	+	+	-	-	+	+	-	-	+	-	+	+	-	-	-	-	+	+	-	-	+	+	+	+
local irradiation	-	-	+	+	+	+	-	-	+	+	-	-	+	-	+	+	-	-	-	-	+	+	-	-	+	+	+	-
other than																												
splenic																												
irradiation																												
steroids	+	+	-	+	+	-	+	-	-	-	-	+	+	-	-	+	+	+	+	-	-	+	+	-	+	+	+	+
blood transfusion	+	+	-	+	-	+	-	+	-	-	+	+	+	+	-	-	-	+	-	+	-	+	+	+	+	-	+	+

* denotes the 18 patients studied in the Memorial Hospital, New York.

TABLE 12

BASIC FORMS OF TREATMENT IN 27 PATIENTS WITH SYMPTOMATIC NON-IMMUNE

HAEMOLYTIC ANAEMIA

Patient	24a	24b	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50
	EB	EB	BC	JC	TD	EF	EG	JH	MG	AH	WH	EH	CM	SM	HM	WQ	MR	JR	PR	MR	AR	AS	RS	NS	US	FT	GV	AW
Chemotherapy	-	+	+	+	-	+	+	-	-	-	-	-	+	+	+	+	+	-	+	+	-	+	-	-	+	-	+	-
Local irradiation other than splenic irradiation	-	-	+	+	+	+	-	-	+	+	-	-	+	-	+	+	-	-	-	+	+	+	-	-	+	+	+	-
Steroids	+	+	-	+	+	-	+	-	-	-	-	+	+	-	-	+	+	+	+	-	+	+	+	-	+	+	+	+
Blood transfusion	+	+	-	+	-	+	-	+	-	-	+	+	+	+	-	+	-	+	-	+	-	+	+	+	+	-	+	+

* denotes the 18 patients studied in the Memorial Hospital, New York.

several different therapeutic measures were being applied, it was considered that steroids had been successful in raising the haemoglobin level and inducing a sustained remission from anaemia lasting six months or more in only two patients (Cases 29 and 48). Both of these received prednisone 40 mg. per day initially, reducing over a two month period to 5-10 mg. per day, and in both the haemoglobin improved by more than 15% above the initial value. One patient (Case 50) developed severe diabetes mellitus while taking prednisone, after which she rapidly deteriorated and died in two months.

4. 6. Observations suggesting implication of the spleen in the haemolytic process in some patients.

The first two patients (Cases 24 and 40) studied in this series, showed evidence that the spleen might be implicated in the haemolytic process, and because their management stimulated further interest in symptomatic non-immune haemolytic anaemia, and because each had some unusual clinical and haematological features, they will be described in further detail.

Case 24. E.B., illustrating the effect of splenic irradiation.

This patient had had pulmonary tuberculosis in 1949, the diagnosis being based on infiltration of the upper zone of the left lung, with possible cavitation, and the recovery of acid fast bacilli from gastric lavage. Anti-tuberculous chemotherapy/

chemotherapy was given in the form of streptomycin and PAS for six months, she recovered well and during subsequent follow-up the pulmonary lesion became calcified and the patient's name was removed from the Tuberculosis Register in 1956. Within two to three weeks (November 1956) she began to deteriorate, complaining of fatigue, loss of appetite, loss of weight and breathlessness. The appearance of a soft homogenous opacity in the upper zone of the left lung (in the area of the previous lesion) indicated recurrence of pulmonary tuberculosis. This relapse was associated with high fever, small lymph glands in most node bearing areas, splenomegaly and high E.S.R. (the first record was 115 mm. in the first hour). There was marked anaemia, Hb. 50%, normal white cell count (7,400) and normal differential count. The bone marrow aspirate was also normal and later grew no tubercle bacilli on culture. A large (2 cm.) supraclavicular gland was biopsied; this showed the typical histological features of caseating tuberculosis, a few tubercle bacilli being present.

Treatment with streptomycin, isoniazid and PAS was started forthwith. There was rapid subjective improvement, the lung lesion cleared and the spleen became impalpable; the temperature did not, however, settle for six weeks and after three months the haemoglobin level was still in the range 60-70%. During this period the white cell count had been gradually rising; it was now 25,000 and primitive granular/

granular cells were appearing in the circulation. Over the next six weeks, despite continuance of chemotherapy, the spleen which once regressed became quite large, the white cell count increased to 58,000, the platelet count also increased into the region of 1 million, and the peripheral blood picture was entirely compatible with chronic myeloid leukaemia. The marrow which had only four months earlier been normal was now also consistent with this diagnosis.

The patient came under the observation of the writer in June 1957 when aged twenty-six years. The clinical and haematological picture had shown no important change over the previous three months. Marrow could not now be aspirated from the sternum or ilium, however, and bone marrow trephine showed definite evidence of myelofibrosis. It was concluded that the patient was suffering from secondary myelofibrosis consequent on the healing of miliary tuberculosis. (This rare complication is well founded in autopsy experience, Crail, Alt and Nadler 1948, but does not appear to have been previously encountered in life). The question of myeloid leukaemia could not be definitely excluded but seemed unlikely in view of the marrow transformation and less likely when the patient's leucocytes were later shown to have no Philadelphia chromosome and to have a normal content of alkaline phosphatase (by courtesy of Dr. A. G. Baikie, M.R.C. Unit, Western General Hospital, Edinburgh).

Anti-tuberculous chemotherapy was continued and the patient/

patient remained moderately well for a further two months. The patient then became ill with high fever, enlarging spleen and anaemia, the haemoglobin level falling to 40%. After a 2.5 litre blood transfusion, the haemoglobin level improved to 80% but fell again to 66% after only five days. A further 6 litres were given in the next three months because of recurring anaemia but the haemoglobin level was raised above 50% for only temporary periods. There was, during this time, no reticulocytosis and no acholuric jaundice but in November 1957 the $T_{1/2}Cr$ was 9 days confirming the presence of a severe haemolytic process.

It was decided to try the effect of irradiation of the spleen. This was supervised by Dr. J. Newall, Consultant Radiotherapist, Royal Infirmary of Edinburgh. A total of 625 rads. were given through an anterior port to cover most of the surface area of the large spleen. The patient's haemoglobin level fell to 49% from 65% in the week after this treatment and a 1.5 litre blood transfusion was given. Thereafter a most remarkable effect was observed; the patient's spleen became quickly smaller, being only just palpable under the right costal margin and the haemolytic process was completely arrested. The patient remained well for four-and-a-half years until June 1962 (p. 153), the haemoglobin level persisting constantly in the region of 80%. The only abnormal haematological features were a tendency to leucocytosis with occasional myelocytes and an excess/

excess of basophils in the circulation and thrombocythaemia, the platelet count reaching levels of 1.5 to 2 million per c.mm. The thrombocythaemia was successfully controlled with radioactive phosphorus which the patient received in 3 mc. amounts in August 1959, August 1960 and September 1961.

Case 40. J.R., illustrating the effect of splenectomy.

This patient was first seen in November 1955, following increasing disability from tiredness, breathlessness, weight loss and generalised aches and pains in the previous four years. She was found to be anaemic, Hb. 64%, the peripheral blood showing a leucoerythroblastic picture and there was moderate splenomegaly. Several attempts to aspirate bone marrow were unsuccessful and a presumptive diagnosis of myelofibrosis was made; however, subsequent marrow trephine (and later autopsy) showed that the marrow was normally cellular and the condition could more correctly be described as "primary myeloid metaplasia" (Bowdler and Pranker 1961). A duodenal ulcer also demonstrated could not be implicated in the anaemia.

In February 1956, the haemoglobin level fell to 39% and blood transfusion was given. Afterwards transfusion was required at shortening intervals and by August of that year the patient was requiring approximately 4 litres of blood per month. At the end of July 1956 it had been decided to irradiate the spleen; a dose of 400 rads. was given/

given through an anterior port and although considerable shrinkage of the spleen was achieved (and the platelet count fell from 110,000 to levels of 40-60,000 per c.mm.), no remission in the haemolytic process was observed. Despite the continuing large transfusion requirement, there was only transitory increase in urobilinogenuria, the serum bilirubin remained in the normal range (the highest value was 0.7 mg./100 ml.) and there was no reticulocytosis.

In August 1956 the red cell survival time was measured and gave a $T_{1/2}^{Cr}$ of 12 days; a typical peripheral blood picture at this time was as follows: Hb. 67%; R.B.C. 3.92 m.; C.I. 0.85; P.C.V. 34%; M.C.V. 86.7 c. μ .; M.C.H.C. 29.1%; W.B.C. 7,800 (occasional myelocytes and normoblasts were present but otherwise the differential count was normal); reticulocytes < 1%; platelets 55,000 per c.mm.

Prednisone therapy was used in the next six weeks, initially 60 mg. daily for three weeks and gradually reducing the dose to 15 mg. daily in the succeeding three weeks. This was without any effect on the rate of fall of the haemoglobin level or on the transfusion requirement. During this period epigastric pain strongly suggested activation of the patient's duodenal ulcer.

It was decided to proceed to splenectomy which was undertaken in September 1956. This was complicated by haematoma/

haematoma formation under the left diaphragm, which had to be drained, but there was no doubt that the haemolytic process was significantly reduced by operation. Only 1 litre of blood was given in October and a further 1 litre in November, and prior to the patient's discharge home at the end of this month a second measurement of red cell survival time showed considerable improvement, the $T_{1/2}^{Cr}$ being 22 days.

The patient remained well for only three weeks, however. Readmission was occasioned by hepatocellular jaundice (which was considered to be due at the time to a serum hepatitis); two weeks later, while liver disease was persisting, she had copious and repeated haemorrhage from the duodenal ulcer. Bleeding continued for three days, repeated transfusion being required, and laparotomy was advised. Partial gastrectomy was undertaken and after initial improvement the patient gradually deteriorated and died in three weeks. The cause of death was generalised peritonitis from dehiscence of the gastro-jejunal anastomosis.

Evidence for a splenic contribution to the haemolytic process using measurements of splenic uptake of ^{51}Cr -labelled red cells.

The experience of the foregoing two patients, together with that in the literature, suggested that at least in some with symptomatic non-immune haemolytic anaemia there might be a splenic component to the haemolytic process. The first/

first (Case 24) had lasting benefit from small doses of splenic irradiation; the second was not helped by radiotherapy but splenectomy was followed by measurable lengthening in red cell survival time. Unfortunately, the latter patient could not be followed for a long enough period to allow useful conclusions. However, the observations in these two patients were the reason for measuring the splenic uptake of ^{51}Cr labelled red cells in twenty-three of the patients with a symptomatic haemolytic process who were subsequently seen.

The results of these measurements are summarised in Table (3). Data listed under Case 24b refer to the further study of this patient (p. 153) when a haemolytic process supervened for the second time, consequent on the transformation of her condition to acute leukaemia.

The observations show a number of features. Firstly there are eight patients (Cases 24b, 27, 32, 35, 41, 44, 47, and 49) in whom the spleen sequestration index at $\text{T}_{\frac{1}{2}}\text{Cr}$ exceeds 100, above which level Jandl and his colleagues (1956) concluded that splenic sequestration was "moderate" to "severe". Secondly there are thirteen patients (Cases 24b, 25, 27, 29, 32, 33, 35, 37, 38, 39, 41, 44 and 49) in whom the spleen/liver ratio exceeds 2.4, above which level Goldberg (1960) indicated that splenectomy was beneficial in acquired haemolytic anaemia. Thirdly, it was only in the eight patients who showed a high value for both of these indices/

TABLE 13

RED CELL SURVIVAL MEASUREMENTS AND DATA REGARDING SPLENIC SEQUESTRATION OF
RED CELLS IN 23 PATIENTS WITH SYMPTOMATIC NON-IMMUNE HAEMOLYTIC ANAEMIA

Patient	24b	25	26	27	28	29	30	31	32	33	35	36	37	38	39	41	43	44	45	46	47	48	49
	E.B. B.C. J.C. T.D. E.F. E.G. J.H. M.G. A.H. W.H. C.M. S.M. H.M. W.Q. M.R. P.R. A.R. A.S. R.S. N.S. U.S. F.T. G.V.																						
$T_{1/2}^{Cr}$ in days	13	21	21	14	20	23	13	22	18	13	9	1.5	22	22	23	22	18	3	13	20	13	18	11
Spleen Sequestration index at $T_{1/2}^{Cr}$ (A)	140	60	50	200	20	60	20	50	240	10	240	30	80	100	80	130	30	170	50	40	110	20	160
Spleen/liver ratio (B)	3.1	2.6	1.73	4.6	2.15	2.65	1.5	2.35	4	2.6	4.4	1.5	4	3.0	3.15	5.7	2.15	3.8	2.4	2.1	2.4	1.7	2.8
Spleen Number (A x B)	434	208	86	920	43	199	30	118	960	26	1056	45	320	300	252	741	65	646	120	84	264	34	448

indices (Cases 24b, 27, 32, 35, 37, 41, 44 and 49) that the Spleen Number (p. 101) exceeded 300; this was the figure above which the only remissions from splenectomy in autoimmune haemolytic anaemia were seen.

The different patterns of uptake of ^{51}Cr labelled red cells by spleen and liver are illustrated by four patients in Fig. 22. Each has a moderate to severe reduction of red cell survival time, but there is clearly a wide spectrum of splenic sequestration of labelled cells. The most rapid destruction of red cells occurs in the first patient (Case 36, $T_{1/2}\text{Cr}$ 1.5 days), an elderly subject with chronic myeloid leukaemia. In him there is no rise in the spleen/praecordium or liver/praecordium ratio during the course of the study, and it must be concluded that neither spleen nor liver is the main site of red cell destruction but that transfused red cells are being removed from the circulation widely throughout the body, presumably by the reticulo-endothelial system. In the second (Case 47) and third patient (Case 35) there is an increasing spleen/praecordium ratio, suggesting increasing importance of splenic sequestration, while in the fourth patient (Case 44) a most dramatic rise in the curve of this ratio against time is seen. Fig. 22 also illustrates the importance of not relying solely on visual interpretation of data. The magnitude of the spleen/praecordium ratio becomes increasingly influenced by the fall in blood radioactivity as the study/

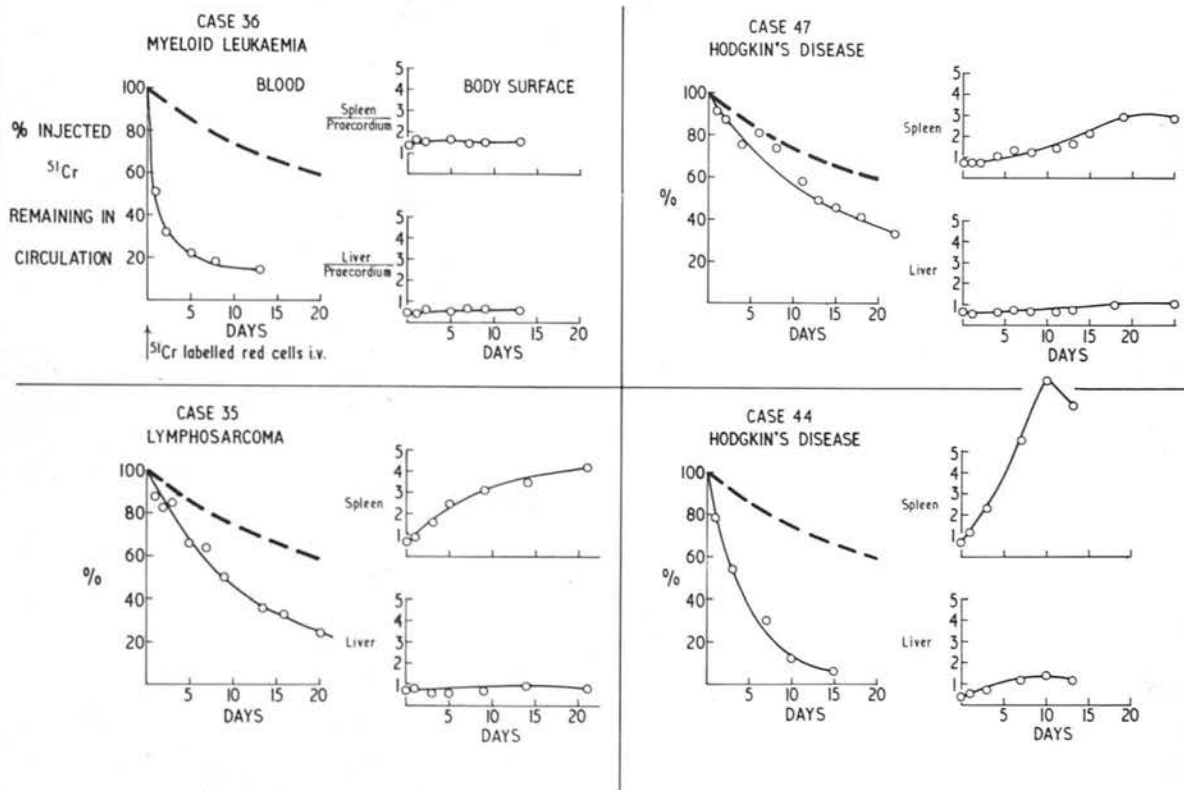


Fig. 22

The range of splenic sequestration of ^{51}Cr labelled red cells in 4 patients with symptomatic non-immune haemolytic anaemia. The best line has been drawn through the data for illustrative purposes.

study continues; hence while the fourth patient (Case 44) appears to have the most severe degree of splenic sequestration, the Spleen Number at T_2Cr in this patient, 646, was actually less than that of the third patient (Case 35) at 1056.

4. 7. Observations on the effect of splenic irradiation on the haemolytic process in patients showing varying degrees of splenic sequestration of red cells.

Opportunity arose for studying the effects of splenic irradiation on the haemolytic process in four of the patients (Cases 24b, 33, 44 and 47) in the present series. The observation was not influenced by other treatment except in Case 24b, who was receiving prednisone in a constant dose of 20 mg. daily at the time. Red cell survival measurement was made in these patients before and after radiotherapy. Because of their different behaviour there follows a brief account of each.

Case 24b. Acute leukaemia. Spleen Number 434.

The course of this patient has been described (p. 144) until June 1962. Her haemoglobin level had remained satisfactory for four-and-a-half years following splenic irradiation and she was now in the eighth month of her first pregnancy. Pregnancy had proceeded uneventfully, although there was some concern for the foetus since conception must have occurred approximately ten days after her last treatment with radioactive phosphorus. Then in June 1962 anaemia/

anaemia suddenly developed, the patient's haemoglobin level falling from 75% to 40% in a period of three weeks.

Following transfusion on two occasions, the patient was delivered of a normal child in mid July and after confinement further blood was required. Transfusion of 3 litres of blood was repeated in mid August and again in mid September. During this period, June to September 1962, there had been a gradual fall in the platelet count from 425,000 to 61,000 and simultaneous increase in the white cell count from 10,500 to 104,000. The latter was associated with the appearance of blast cells in the peripheral blood which now accounted for 92% of the total white cell count; this change in the peripheral blood, together with the appearances in the bone marrow, confirmed that the patient was suffering from acute myelogenous leukaemia.

Steroid therapy was started (initially prednisone in a dose of 40 mg. per day) but this was found to have no effect on the rate of fall of the haemoglobin level and repeated blood transfusions continued to be required. Prednisone was maintained in a dose of 20 mg. per day. The $T_{1/2}Cr$ in October 1962 was 13 days, and because of the earlier good result from splenic irradiation (p. 144) this treatment was tried for a second time, despite the transformation of the underlying disease. A total of 100 rads. was given through an anterior port. This reduced the size of the spleen/

spleen but had no effect on the transfusion requirement and a further measurement of red cell survival two weeks after the end of radiotherapy showed the $T_{1/2}Cr$ to be worse at 5 days (Fig.23). Despite continuance of prednisone and the giving of 6-mercaptopurine the patient died one month later.

Case 47. Hodgkin's disease. Spleen Number 264.

This young woman with Hodgkin's disease had involvement mainly of liver, spleen and para-aortic lymph nodes. The condition had presented with cervical lymph gland enlargement, but there had been no recurrence in this area over a three year period, following local irradiation. The patient's main symptom at the time of investigation was profuse night sweating, the temperature reaching levels of 106-107° F. Anaemia was not prominent (Hb. 81%) but in recent months had usually been in the range 55-70%; however, the $T_{1/2}Cr$ was found to be 13 days. She was treated with a course of bipiperidyl, a new intravenously administered alkylating agent under trial. At the end of this time the $T_{1/2}Cr$ was unchanged (14 days, see Fig.24) and the fever and sweats continued. The patient then received splenic irradiation to a total dose of 2,000 rads. through anterior and posterior ports. This produced marked regression of spleen size, almost immediate correction of pyrexia and some reduction in the haemolytic process. The measurement of $T_{1/2}Cr$ which was started four weeks after the end of radiotherapy showed improvement to 18 days.

Case 44/

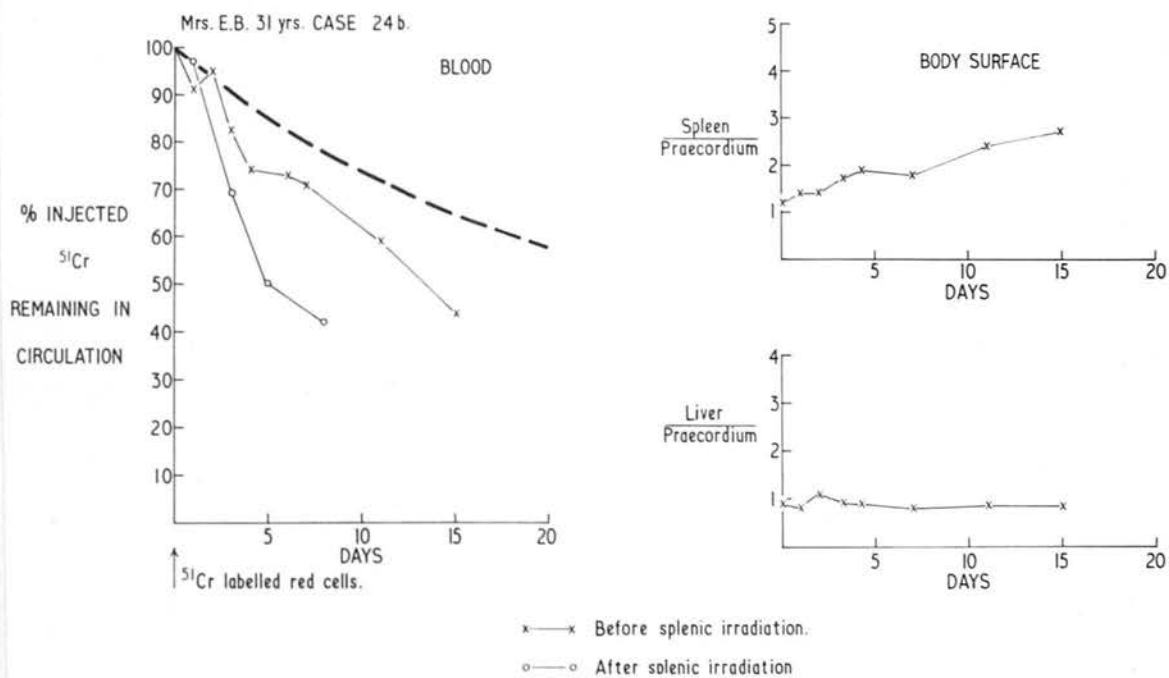


Fig. 23

Red cell survival measurements in Case 24b before and after splenectomy. This patient's body tissues were studied for content of radioactivity at subsequent autopsy (p. 159).

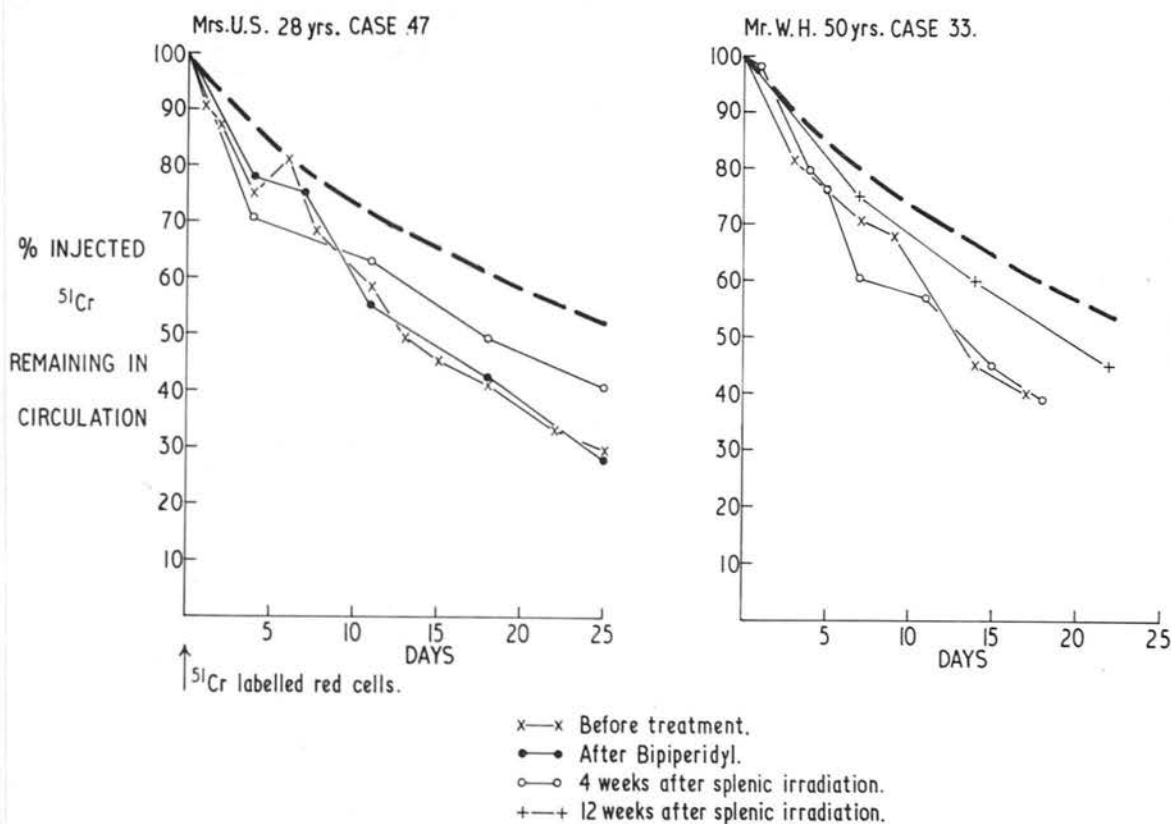


Fig. 24

The effect of splenic irradiation on red cell survival in two patients with symptomatic non-immune haemolytic anaemia. Note the delay in suppression of the haemolytic process in Case 47.

Case 44. Hodgkin's disease. Spleen Number 646.

This patient who had a relatively short history (eighteen months) of illness had already received local irradiation of neck, axillary and groin lymph nodes, chemotherapy (TEM) and steroids prior to the development of an overt haemolytic process. This is the only patient in the series who showed the classical signs of reticulocytosis and acholuric jaundice.

In the three months prior to investigation, she had required 6 litres of blood; the $T_{1/2}Cr$ was found to be markedly shortened, being 3 days. There was evidence of considerable splenic uptake of ^{51}Cr labelled red cells, the spleen sequestration index being 170, the spleen/liver ratio at $T_{1/2}Cr$ being 3.8 and the Spleen Number 646. Accordingly irradiation of the spleen was recommended, a total of 700 rads. being given through anterior and posterior ports. This was succeeded by striking clinical remission, the reticulocyte count and serum bilirubin returned to normal and a further estimate of the $T_{1/2}Cr$, four weeks after the end of radiotherapy, gave a value of 18 days (Fig. 25). During the six months' follow-up that was possible in this patient, no further blood transfusion was required.

Case 33. Myelofibrosis. Spleen Number 26.

This man first attended hospital because of back pains and joint pains in 1959. At that time, he had marked hepatosplenomegaly, hyperuricaemia and radiographic changes in/

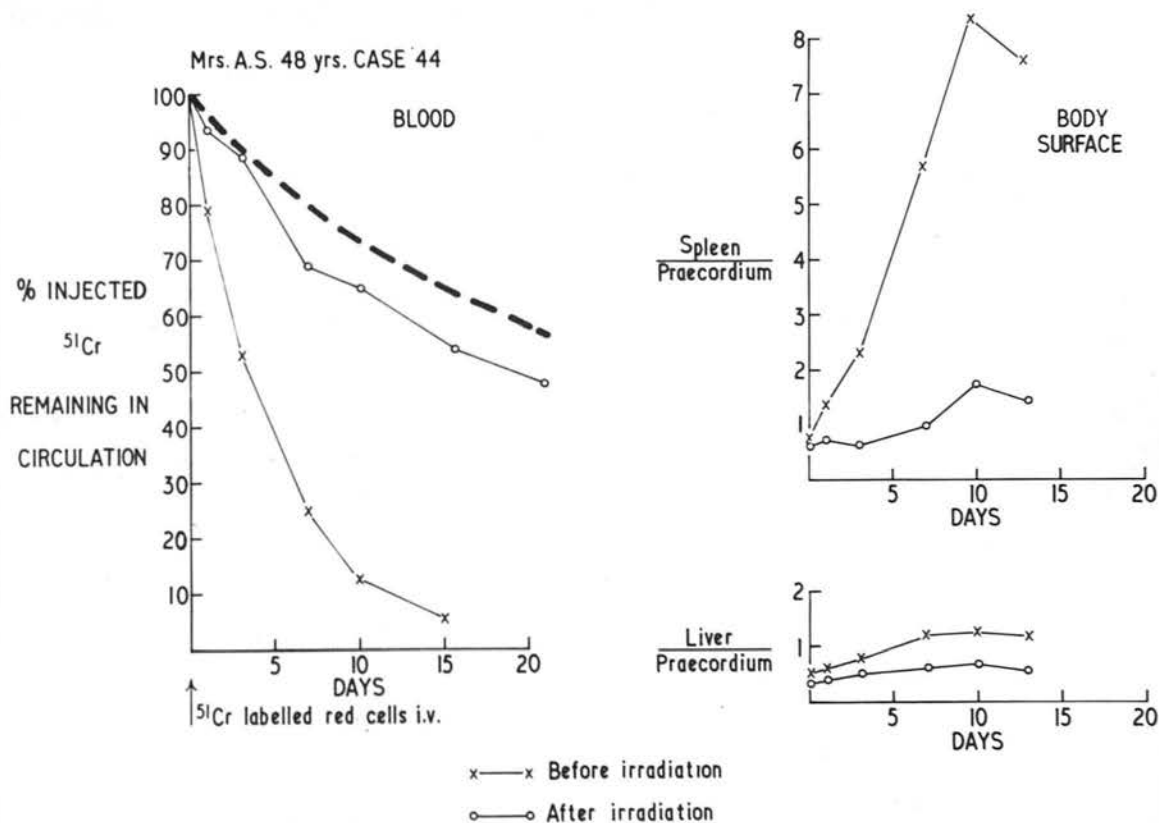


Fig. 25

Satisfactory response to splenic irradiation in a patient with symptomatic non-immune haemolytic anaemia who showed a very high degree of splenic sequestration of ^{51}Cr labelled red cells before treatment.

in the lumbar spine and sacro-iliac joints suggesting early ankylosing spondylitis. The peripheral joint pains were attributed to secondary gout and certainly considerable symptomatic relief was obtained in the subsequent three years from the use of uricosuric agents, notably colchicine and "Anturan". Haematological investigations revealed a moderate pancytopenia, Hb. 70-80%, primitive white cells and red cells in the peripheral blood, and after failure to obtain marrow, the diagnosis was shown to be myelofibrosis.

The patient remained in a steady state, both clinically and haematologically, for two-and-a-half years; then in January 1962 he began to complain of increasing fatigue, diarrhoea and weight loss. Detailed investigations failed to reveal any new development other than the anaemia. This was treated empirically with iron, vitamin B₁₂ and folic acid without effect and blood transfusion became necessary. The transfusion requirement increased over the next seven months, at the end of which time the patient was receiving 2 to 2.5 litres per month.

In August 1962 there were none of the usual signs of haemolytic disease but red cell survival measurement showed the T_{1/2}Cr to be considerably reduced at 13 days. Despite the lack of evidence of splenic sequestration (Spleen Number 26, see Table 13), it was decided to try the effect of splenic irradiation. A total of 125 rads. was given through an anterior port at the end of August. There seemed/

seemed at first to be acceleration of the haemolytic process in that the patient's haemoglobin level fell to 50% on 11.9.62 and 2 litres of blood were given, then to 40% on 2.10.62 when a further 2.5 litres were required. A second measurement of $T_{1/2}^{51}\text{Cr}$ at this time gave the same figure as that obtained before treatment, namely 13 days. Subsequently no further transfusion has been required during the past four months to the time of writing (28.2.63) and re-assessment of the red cell survival time in December 1962 showed improvement in the $T_{1/2}^{51}\text{Cr}$ to 18.5 days, (Fig. 24)

Comment on the patients receiving splenic irradiation.

(i) Of the four patients treated by splenic irradiation, three have shown significant improvement in red cell survival time and have received clinical benefit. One patient (Case 24) who had acute leukaemia did not respond, the $T_{1/2}^{51}\text{Cr}$ being actually less after treatment than before.

(ii) The impression had been gained from earlier experience (p. 147) that if the haemolytic process were to respond to splenic irradiation this might not occur immediately. In one patient (Case 33) the transfusion requirement increased immediately following radiotherapy and, although improvement in red cell survival occurred subsequently, no change in $T_{1/2}^{51}\text{Cr}$ was found in the immediate post-irradiation period.

(iii) The response to splenic irradiation bore no relation/

relation to the splenic uptake of ^{51}Cr labelled red cells. The result in Case 33 with a low Spleen Number of 26 appears to be just as good as that in Case 44, Spleen Number 646.

4. 8. The uptake of ^{51}Cr labelled red blood cells by different body tissues measured at autopsy related to in vivo assessment of the splenic role in the haemolytic process.

Two patients (Cases 24 and 49) in the present series died at a short enough interval after a red cell survival study with chromium-51 to permit measurement of the concentration of radioactivity in various body tissues obtained at autopsy. The interval between the administration of radioactive chromium and death was 36 days in Case 24 and 21 days in Case 49. The data regarding red cell survival and body surface measurements have already been illustrated for Case 24 in Fig. 23; those for Case 49 are shown in Fig. 26. Table 13 gives the figures for spleen sequestration index, spleen/liver ratio and Spleen Number. The radioactivity in the different tissues per unit of wet weight for the two patients are summarised in Table 14. In each case the radioactivity of a given tissue was obtained in a weighed amount (approximately 5 g.), using a well type scintillation counter, and measurement continued until a total of at least 5,000 'counts' was registered.

In Case 24 all but three tissues were found to give 40 or less 'counts' per minute per g. For the stomach the figures was 80, for the liver 140 and for the spleen much/

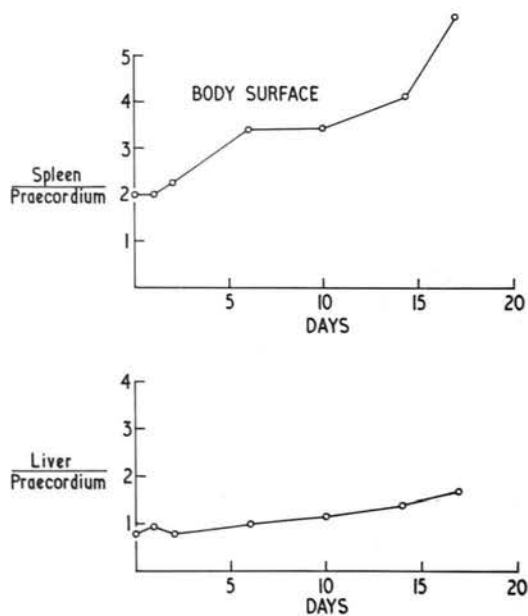
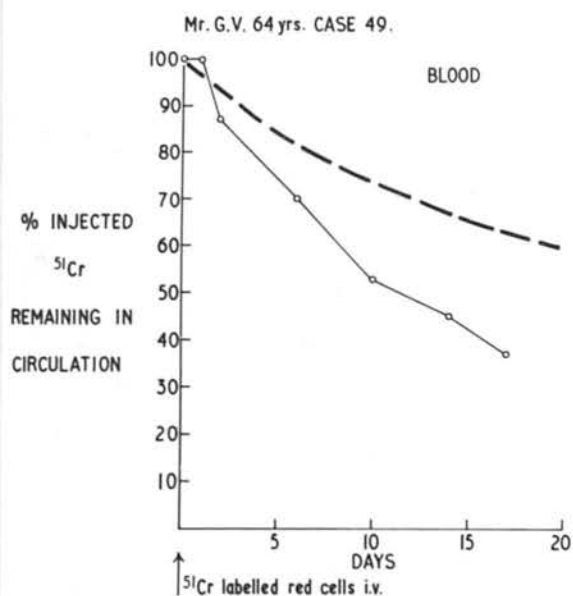


Fig. 26

The data obtained regarding red cell survival and splenic and liver uptake of radioactivity in Case 49. This patient's tissues were subsequently analysed for content of radioactivity at autopsy (p. 159).

TABLE 14

THE CONTENT OF RADIOACTIVITY IN DIFFERENT BODY TISSUES
AT AUTOPSY FOLLOWING RED CELL SURVIVAL MEASUREMENT WITH
 ^{51}Cr LABELLED RED CELLS IN TWO PATIENTS WITH SYMPTOMATIC
NON-IMMUNE HAEMOLYTIC ANAEMIA

Counts of Radioactivity per Minute per Gm. Wet Weight of Tissue			
Case 24 : Acute leukaemia		Case 49 : Lymphosarcoma	
Gall bladder	4	Bladder	24
Ileum	7	Skin	30
Large bowel	8	Prostate	32
Skin	8	Stomach	39
Jejunum	9	Thyroid	42
Heart	11	Small bowel	44
Pancreas	11	Pancreas	49
Marrow	12	Duodenum	50
Lung	14	Large bowel	53
Brain	15	Heart	57
Oesophagus	20	Gall bladder	71
Kidney	22	Lung	80
Bladder	25	Suprarenal	85
Thyroid	27	Lymph node	87
Lymph node	28	Kidney	89
Suprarenal	37	Retroperitoneal tumour	145
Uterus	40	Oesophagus	150
Stomach	80	Liver	158
Liver	140	Marrow	1050
Spleen	694	Spleen	1100

much the highest, 694. When consideration is given to the weight of liver and spleen, the total organ content of radioactivity can be calculated. The former weighed 2115 g. and the latter 1100 g.; hence the total amount of radioactivity in the liver was 296100 counts per minute and in the spleen 763400 counts per minute. This gives a spleen/liver ratio of 3.58 which compares favourably with the spleen/liver ratio at $T_{1/2}Cr$ in vivo of 3.1.

In Case 49 a different pattern of uptake is seen. In most tissues there is a small amount of radioactivity ranging from 24 to 89 counts per minute. The liver, oesophagus and retroperitoneal tumour showed a similar uptake of radioactive labelled cells (145-158 counts per minute); the presence of oesophagus in this intermediate group may have been due to invasion by contiguous mediastinal tumour. The spleen contained much more radioactivity than these other tissues with 1100 counts per minute. The significant feature in the study of this patient was the equally high amount of radioactivity in the bone marrow.

In Case 49 the liver weighed 2350 g. and the spleen 750 g. Hence the total amount of radioactivity in these organs was respectively 371300 counts per minute in the liver and 825000 counts per minute in the spleen. These data give an actual spleen/liver ratio of 3.22 compared with the in vivo measurement at $T_{1/2}Cr$ of 2.8.

It/

It is not possible to estimate the total amount of radioactivity in the bone marrow in this patient. Mechanik (1926) found that the bone marrow accounted for 3.4 to 5.9% of body weight or 1600 to 3700 g. Assuming a marrow weight, arbitrarily, of 2500 g. then the marrow in Case 49 would have contained 2625000 counts per minute of radioactivity at the time of death which is approximately four times more than that found in the spleen. These data suggest that in some patients the marrow may well be a more important site of erythroclasis than any other tissue in the body, a possibility already indicated by animal experiment (Meischer 1956, Ehrenstein and Lockner 1958).

4. 9. Main conclusions from studies of patients with symptomatic non-immune haemolytic anaemia.

(i) In this group of twenty-seven patients with symptomatic non-immune haemolytic anaemia, recognition of the haemolytic process has depended solely on estimation of the red cell survival time. The $T_{1/2}Cr$ has varied widely in the range 1.5 days to 23 days, with a mean value of 15.8 days. In leukaemia, reticulosis and myeloid metaplasia, conditions which offer multiple causes for anaemia, it has been considered to be unlikely that increased red cell destruction is more than a contributory factor to a low haemoglobin level when the $T_{1/2}Cr$ exceeds 20 days. However, in seventeen patients the $T_{1/2}Cr$ was less than 20 days and in ten/

ten of these it was considerably shortened, being less than 15 days. Despite marked increase in the rate of red cell destruction in these latter patients, only one in the entire series showed the classical signs of an overt haemolytic process, acholuric jaundice and reticulocytosis.

(ii) From the management of the first two patients in the series, the impression was gained that the spleen might be implicated in the haemolytic process, at least in some. The first, who had a large transfusion requirement maintained a satisfactory haemoglobin level for four-and-a-half years without the need for further blood after splenic irradiation; the second showed correction of the red cell survival time ($T_{1/2}Cr$ of 12 days improving to $T_{1/2}Cr$ of 22 days) following splenectomy but died shortly afterwards from post-operative complications, consequent on gastrectomy and long follow-up was not possible.

(iii) The uptake of radioactive labelled cells by the spleen was studied subsequently in twenty-three patients. It was found that the degree of sequestration of ^{51}Cr labelled red cells could be just as great in this group of patients as had been encountered in the patients with autoimmune disease. Seven patients were found to have a Spleen Number in excess of 300.

(iv) After the initial experience referred to in (ii), four additional observations were made of the effect of splenic/

splenic irradiation on the haemolytic process with measurements of the red cell survival time before and after treatment. Three patients (two with Hodgkin's disease and one with myelofibrosis) showed clinical and haematological improvement for periods of observation up to six months; the fourth patient who had acute leukaemia was not benefited, the $T_{1/2}^{51}\text{Cr}$ being 5 days after treatment compared with 13 days before.

The response to splenic irradiation in these patients bore no relation to the assessments of splenic involvement in sequestering ^{51}Cr labelled red cells. Case 33, whose Spleen Number was 26, did just as well as Case 44 whose Spleen Number was 646. No explanation is available for this finding. It is, however, possible that in addition to affecting haemolysis by reducing the size of the spleen, splenic irradiation may have had indirect effects on erythropoiesis; analogous remote effects on leucopoiesis are the basis for the treatment of chronic leukaemia by radiotherapy. Evidence for interference with red cell production by irradiation is suggested by the course of one patient (Case 33) who had a temporary increase in transfusion requirement in the period immediately after treatment without showing any change in the $T_{1/2}^{51}\text{Cr}$; subsequently the haemoglobin level became steady, no further blood was required and the red cell survival time was found to have improved./

improved.

(v) Finally opportunity arose of measuring the uptake of radioactive labelled red cells by different body tissues in two patients with symptomatic non-immune haemolytic anaemia, who died shortly after red cell survival measurement using radioactive chromium. The data showed reasonable correlation between the spleen/liver ratio of radioactivity at autopsy and the spleen/liver ratio at $T_{1/2}Cr$ obtained earlier during life. However, in the second patient the concentration of radioactivity in the spleen was the same as that in the bone marrow, indicating that the role of the latter in red cell destruction (assuming a marrow weight of 2500 g.) was probably about four times greater than that of the spleen. Since this activity of the bone marrow in erythroclasis cannot be assessed in vivo and since the results in these two patients indicate that there is considerable variation in the sites of uptake of red cells between individuals and between different diseases, the present methods for evaluating the role of the spleen in the haemolytic process in symptomatic non-immune haemolytic anaemia do not form a useful guide to the patient's management.

Experience in this small number of patients suggests that splenic irradiation should be considered in doses up to 400 rads. when there is myeloid metaplasia, and up to 2000/

2000 rads. when the spleen is believed to be infiltrated by a reticulosis, if a patient's anaemia is disabling and there is shown to be a significant contribution from haemolysis ($T_{1/2}Cr$ less than 20 days).

CHAPTER FIVE

CONGENITAL SPHEROCYTOSIS5. 1. Introduction.

Although congenital spherocytosis is encountered infrequently, it is the most important haemolytic disorder, resulting from an intrinsic red cell defect, that occurs in Great Britain. It was probably the first blood disorder in which excessive red cell destruction was recognised to be the mechanism of production of anaemia. Reference has already been made to the most significant early reports, notably that of Vanlair and Masius (1871) and Minkowski (1900) who respectively first described and characterised the condition and later that of Chauffard (1907) who drew attention to the abnormal fragility of the erythrocytes in hypotonic dilutions of saline.

All large studies of congenital spherocytosis have been in Caucasian subjects; however, there have been isolated reports of its occurrence in negroes, Kline and Holman (1957) finding six examples in one American family and Metz (1959) describing two in the South African Bantu.

While the condition is congenital, it cannot always be proved to be familial. A number of investigators have observed patients, neither of whose parents appeared to be affected (Debree, Lamy, See, Schrameck 1938, Race 1942, Young, Izzo and Platzer 1951). Young (1955) found that in five/

five out of twenty-eight families with the disease, the blood of both parents of the propositus could not be distinguished from normal; Race (1942) also reported this finding in four of his twenty-eight families. In the former series, spherocytosis was, however, discovered in a maternal grandmother in one family where the parents of the patient were unaffected, and in a maternal aunt in another. On the other hand, several detailed family trees have been published showing many affected individuals in three and four generations (Gänsslen, Zipperlen and Schüz 1925, Debre, Lamy, See and Schrameck 1938, Young, Izzo and Platzer 1951). The gene for the red cell abnormality in these families appears to behave as a Mendelian dominant transmissible through either parent, although there is usually a deficiency in the expected number of affected siblings. Race (1942) attributed this latter finding to an unusually high miscarriage rate and infant mortality; Race also considered that variation in penetrance and expression of the defective gene was a likely explanation, both for the shortage of siblings with signs of disease and for the high incidence of unaffected parents. Dacie (1960) was also inclined to regard limitation of penetrance in a carrier parent rather than gene mutation as the more likely explanation for instances of "sporadic congenital spherocytosis", but proposed that those patients differing in the mode of inheritance/

inheritance of the disorder may also differ in the nature of the red cell defect, and that there may be more than one variety of congenital spherocytosis.

The sexes have been equally affected in reported series. The condition has been recognised for the first time in the early hours of life (Stamey and Diamond 1957, Burman 1958) and at the other extreme of age, in a seventy-seven year old man, (Race 1942). In the experience of Young and his colleagues (1951) anaemia or jaundice was detected in the first five years of life in 50 per cent of patients and before the age of forty-five years in most of the remainder. It seems highly probable, however, that some affected subjects with mild disease pass through life without the diagnosis ever being made.

Apart from the presence of congenital spherocytosis, the condition is usually characterised by a varying degree of chronic anaemia and icterus. The spleen is almost always enlarged and may be huge (Wintrobe 1961); it was readily palpable in twenty-three of the twenty-eight patients of Young, Izzo and Platzner (1951). Gänsslen and his colleagues (1925) stated that the spleen may not be palpable in 30 per cent of cases, but Wintrobe (1961) considers that this high proportion must have been due to the inclusion of relatives with mild disease and "formes frustes" in these authors' series.

Two aspects of congenital spherocytosis require special mention. The first is the "haemolytic crisis". This may occur at any time, even for the first time in late adult life. It is an acutely developing illness and anaemia, with prostration, vomiting, fever, tachycardia and abdominal pain, often simulating an acute abdominal surgical emergency. The episodes have been attributed to sudden acceleration of red cell destruction, but Owren (1948) suggested that the mechanism was more likely to be acute bone marrow arrest with paradoxical cessation of haemolysis; this was based on the return of serum bilirubin and urine urobilinogen values to normal, and sudden reticulocytopenia, thrombocytopenia and leukopenia and acute erythroid hypoplasia in studies in four cases at the height of the crisis. Dameshek (1941) had earlier suggested that the crisis might be produced by a combination of increased haemolysis and bone marrow inhibition, consequent on increased splenic activity. This view was reaffirmed in a later report (Dameshek and Bloom 1948).

The second is biliary tract disease. Cholelithiasis from increased bile pigment production has been found to have a very high incidence in large series of patients, varying from 43 per cent (Bates and Brown 1952) to 85 per cent (Young, Izzo and Platzer 1951). Gairdner (1939) reported gall stones in a three year old child.

Quite apart from the disability from chronic anaemia, the/

the possibility of these two complications, haemolytic crisis and gall stone production, both of which are potentially serious, is the main indication for definitive treatment of the haemolytic process (p. 194).

A number of developmental abnormalities have been described in different series of patients (Gänsslen, Zipperlen and Schütz 1925, Hansen and Klein 1934). The most common of these are tower shaped skull, epicanthus, misplaced teeth, polydactyly and brachydactyly. An uncommon complication which may be the chief complaint and which has no known explanation is chronic leg ulceration, just above the malleoli (Eppinger 1930, Taylor 1939). In other untreated patients a chronic dermatosis may persist in the same situation (Beinhauer and Gruhn 1957).

The precise nature of the red cell defect in congenital spherocytosis has still to be established. That the haemolytic process is due solely to a corpuscular abnormality has been suspected since the time of Naegeli (1931), and was conclusively shown in the pioneer cross transfusion experiments of Dacie and Mollison (1943); these latter investigators demonstrated that normal erythrocytes had a normal survival time in the circulation of recipients with congenital spherocytosis, but that cells from a patient with the condition were destroyed rapidly in the circulation of a healthy recipient (with a spleen).

The/

The rapid spontaneous haemolysis of spherocytes in vitro gave the first indication of a biochemical abnormality (Ham and Castle 1940, Dacie 1941). Later Selwyn and Dacie (1954) and Selwyn (1955) demonstrated that the addition of glucose retarded the corpuscular potassium loss and reduced, but did not abolish, this excessive autohaemolysis in vitro; Young, Izzo, Altman and Swisher (1956, 1958) then found that the effect of glucose could be neutralised by the addition of inhibitors of glycolysis, e.g. iodoacetate, and that a number of purine ribosides were even better than glucose in reducing haemolysis.

More recently, using radioisotope and chromatographic techniques, Prankerd, Altman and Young (1955, 1957, 1960) have demonstrated abnormalities of intracorpuseular glycolysis; these investigators found, using phosphorus-32, that there is a smaller flux of labelled phosphorus into adenosine triphosphate and into 2,3-diphosphoglyceric acid than in normal cells at the same time as there is increased flux into inorganic orthophosphate. These observations have been confirmed (Motulsky, Giblett, Coleman, Gabrio and Finch 1955). Since 2,3-diphosphoglycerate, one of the triose phosphates, accounts for as much as 50 per cent of the total acid soluble organic phosphate in the red corpuscle and is an important store of energy (Hughes-Jones and Robinson 1957), the defect in phosphorylation in congenital spherocytosis can be seen as a most likely cause of reduced red/

red cell survival. Possible enzyme deficiencies to explain these observations have been postulated by Prankerd (1959) but have yet to be proved.

Allusion has already been made (p. 11) to changes in the phospholipid component of the spherocyte membrane which might have even greater importance than disordered glycolysis in the genesis of the haemolytic process; whatever the fundamental defect (or defects) is found to be, the consequence is that the red corpuscles become increasingly spheroidal with senescence and have a very much reduced survival time in the patient's circulation. The importance of recognising congenital spherocytosis lies in the universally good effect of splenectomy in arresting excessive red cell destruction and in producing clinical cure of the disability.

5. 2. Composition of the group of patients with congenital spherocytosis and their main clinical features.

The data considered in this section are summarised in Table 15.

Twenty-six patients with congenital spherocytosis have been studied; eight of these were members of one family. There were fourteen males and twelve females and the age distribution at the time of diagnosis ranged from 18 months to 62 years.

The various modes of presentation will be discussed later/

TABLE 15

COMPOSITION OF A GROUP OF 26 PATIENTS WITH CONGENITAL SPHEROCYTOSIS AND THEIR
PRINCIPAL CLINICAL FEATURES AT THE TIME OF DIAGNOSIS

Patient	Age	Sex	Possible Duration of Symptoms	Mode of Presentation	Family History of Disease at Time of Diagnosis	Main Clinical Features at Time of Diagnosis		
						Pallor	Icterus	Degree of Splenomegaly
51. G.B.	19	M	9 weeks	Jaundice	No	0	+	
52. J.C.	21	M	3 years	Symptoms of anaemia Left upper abdominal pain	Yes	+	+	
53. F.C.	42	M	2 weeks	Acute anaemia following upper respiratory tract infection	Yes	+	+	
54. J.C.	7	M	1 week	Acute anaemia at same time as sister (Case 55)	Yes	+	+	
55. M.C.	4	F	4 days	Acute anaemia at same time as brother (Case 54)	Yes	+	±	Not palpable
56. M.C.	25	M	Many years	Intermittent abdominal pains, dyspepsia and jaundice since childhood	No*	0	+	
57. A.C.	23	F	12 years	Incidental finding of splenomegaly. Intermittent anaemia subsequently	No	+	+	
58. M.F.	62	F	4 years	Acute attack of upper abdominal pain and jaundice	No*	0	0	
59. W.F.	38	M	-	Moderate anaemia subsequently Discovered following recognition of disease in mother No symptoms	Yes	0	0	
60. E.H.	42	F	20 years	Chronic anaemia. Acute attack of abdominal pain and vomiting 2 weeks prior to diagnosis	No	+	+	
61. G.I.	20	M	8 years	Recurring jaundice associated with symptoms of anaemia	No	0	+	
62. B.K.	40	F	28 years	Relapsing jaundice age 12 years Return of jaundice in 9 months prior to diagnosis with symptoms of anaemia	No	+	±	Not palpable
63. R.M.	12	F	11 days	Acute anaemia with abdominal pain and vomiting. Backwardness at school	No	+	+	
64. A.M.	57	M	-	Discovered in survey of relatives of Case 67. Pernicious anaemia (subsequently confirmed) diagnosed 10 years earlier	Yes	0	0	
65. D.M.	48	M	1 year	Recurrent biliary obstruction and cholangitis following cholecystectomy 1 year earlier	No*	+	+	
66. C.M.	18	F	1 month	Anaemia during second pregnancy	No*	+	0	
67. M.R.	52	F	13 years	Recurring jaundice and anaemia	No*	+	+	
68. P.R.	6	F	2 weeks	Acute anaemia	No	+	0	
69. H.S.	18 months	F	-	Discovered in family survey (Case 71)	Yes	0	0	
70. J.S.	2½	M	-	Discovered in family survey (Case 71). Mongol	Yes	±	±	
71. M.S.	36	F	7 weeks	Splenic infarction	Yes	0	±	
72. N.S.	16	M	2 years	Recurrent jaundice. Anorexia, fever and dark urine during 2 weeks prior to diagnosis	No (Parents not known)	+	+	
73. S.S.	5	F	-	Discovered in family survey (Case 71)	Yes	0	0	
74. D.W.	10	M	-	Discovered in family survey (Case 75)	Yes	0	0	
75. G.W.	48	M	1 week	Acute anaemia leading to angina	No*	+	+	
76. W.W.	29	M	3 years	Cholecystitis. Cholecystectomy Obstructive jaundice with cholangitis	No	+	+	

* Relatives found to show spherocytosis on subsequent investigation.

later (p. 177) but the duration of symptoms ranged widely from a few days (Cases 54, 55, 63, 68 and 75) to many years (Cases 56, 57, 60, 62 and 67). In the latter group were patients who had followed a chronic relapsing course (e.g. Case 60) and others who had had symptoms in childhood which were attributable to the disease, followed by a long period of good health, prior to the illness which led to diagnosis (e.g. Case 62).

Only eleven patients had a definite family history of the condition at the time of diagnosis, but six others were discovered to have affected relations in subsequent studies. Further consideration is given to the data on inheritance of disease in this series of patients on page 204.

There was a considerable degree of pallor and icterus at the time of first examination. Fourteen patients were considered to be anaemic while twelve were definitely jaundiced; in another four patients jaundice was doubtful. The spleen was definitely palpable in all but two patients (Cases 55 and 62), being usually enlarged approximately 5 - 15 cm. below the left costal margin; however, in two patients (Cases 60 and 76) the spleen tip extended to the level of the umbilicus. In the two patients not showing clinical splenomegaly the spleen size was assessed subsequent operation. In both, the organ was enlarged; in Case 55, a child of four years who presented after a haemolytic crisis, the organ weighed 139 g. and would only have been/

been palpated with difficulty; in Case 62 the spleen was stated to be three times normal size and would have been palpable but for a rather obese abdominal wall.

A number of miscellaneous clinical features are worthy of mention. The only developmental defect noted in the series of patients was in Case 61 who had a hare lip. Amentia was encountered twice; Case 63 is a high grade mental defective capable of a routine type of work in a biscuit factory, Case 70 is a mongol requiring institutional care. Cases 57 and 60 have each had three miscarriages and have childless marriages. Case 64 has Addisonian pernicious anaemia; this developed ten years before the patient was recognised to have congenital spherocytosis and has been confirmed by the finding of histamine fast gastric achlorhydria and malabsorption of radioactive cobalt-labelled vitamin B₁₂, which is corrected by the giving of intrinsic factor.

Chronic leg ulceration was not encountered in any of the patients; however, in two (Cases 62 and 65) a chronic dermatosis was present. In Case 65 the condition was a scaly psoriatic type of lesion which encircled the lower one third of each leg above the malleoli. In Case 62 there was a papulo-erythematous eruption and atrophy in the skin on the medial aspect of each leg above the internal malleolus. This is illustrated in Plate 7.



Plate 7

Case 62 Congenital spherocytosis.
Chronic dermatosis affecting the legs.

5. 3. Main haematological and biochemical features at the time of diagnosis

The data referred to in this section are summarised in Table 16.

The haemoglobin level at the time of diagnosis ranged from 18% (Case 54) to 95% (Case 59); the former presented after a haemolytic crisis, the latter was discovered after the condition had been recognised in his mother at the age of sixty-two years; he was thirty-eight years old and had never had any symptoms of disease. The mean value for the haemoglobin level was 64.6%. The reticulocyte count was not significantly raised in five patients; in Case 53 conditioned folic acid deficiency was probably present (p. 181); Cases 54 and 55, a brother and sister, developed a haemolytic crisis within days of each other and were first seen with profound anaemia (p. 179), they did not, however, show signs of aplasia or maturation arrest on marrow examination; Cases 56 and 59 only had mild anaemia when first seen. In the remaining patients, the reticulocyte count ranged from 4.5 to 29%, the mean value being 12.2%.

Measurements of E.S.R. are available in all but six patients. In contrast to the finding in idiopathic autoimmune haemolytic anaemia (p. 68) marked elevation of the E.S.R. appears to be uncommon in the patients with congenital spherocytosis, being above 50 mm. in only two (the brother and sister who presented with profound anaemia after a/

TABLE 16

MAIN HAEMATOLOGICAL AND BIOCHEMICAL FEATURES IN 26 PATIENTS WITH CONGENITAL SPHEROCYTOSIS AT THE TIME OF DIAGNOSIS

Patient	Hb%	Retic% E.S.R.	Other Features in Peripheral Blood	Osmotic Fragility of Red Cells per cent Na Cl.			Bone Marrow	Serum Bilirubin	Urobilinogenuria	Blood Group	T _{1/2} Cr in days
				Onset of Haemolysis	End of Haemolysis	50% Haemolysis					
11. G.B.	91	12.7	4 Spherocytosis	0.70	0.35	0.490	Normoblastic hyperplasia	3.4	++	-	9
12. J.C.	61	17.3	15 Spherocytosis	0.75	0.40	0.515	Normoblastic hyperplasia initially Megaloblastic erythropoiesis developing subsequently	4.5	+	A+ve	10
13. F.C.	32	2.2	14 Spherocytosis Leucopenia and thrombocytopenia	-	-	-	Megaloblastic erythropoiesis	1.5	+++	O+ve	-
14. J.C.	18	2	75 Spherocytosis Normoblasts	0.72	0.48	0.595	Normoblastic hyperplasia	-	+	O+ve	-
15. M.C.	37	< 1	55 Spherocytosis	0.72	0.52	0.610	Normoblastic hyperplasia	1.4	++	O+ve	-
16. M.C.	80	1.6	- Normal	0.55	0.38	0.440	-	-	+	-	-
17. A.C.	63	7.4	33 Spherocytosis	-	-	-	Normoblastic hyperplasia	2.5	+	-	12
18. M.F.	80	6.5	6 A few microspherocytes	0.55	0.46	0.535	-	0.5	+	O+ve	14
19. W.F.	95	1.9	- Normal	0.55	0.40	0.495	-	-	-	-	-
20. E.H.	65	12	5 Aniso-poikilocytosis but no definite microspherocytosis	0.70	0.50	0.60	-	2.3	++	-	6.5
21. G.I.	81	10.2	- Spherocytosis	0.90	0.46	0.520	-	3.9	+	B+ve	-
22. B.K.	65	15	14 Spherocytosis	0.75	0.40	0.525	Megaloblastic erythropoiesis	0.7	-	B+ve	5.5
23. R.M.	50	26.5	42 Spherocytosis Normoblasts	0.85	0.35	0.545	-	0.8	++	O+ve	-
24. A.M.	94	4.5	- Aniso-poikilocytosis	0.44	0.30	0.370	-	1.7	+	A+ve	16
25. D.M.	75	7	- Spherocytosis	-	-	-	Normoblastic hyperplasia	3.7	+	O+ve	-
26. C.M.	76	9.2	10 Spherocytosis	-	-	-	Normoblastic hyperplasia	1.2	+	O+ve	8
27. M.R.	79	7.8	2 Spherocytosis	0.55	0.42	0.435	-	2.4	+	A+ve	13
28. P.R.	23	19	10 Spherocytosis Normoblasts	0.70	0.40	0.545	-	1.4	0	O+ve	-
29. H.S.	80	7	4 Spherocytosis	-	-	-	-	-	+++	O+ve	-
30. J.S.	47	9.5	5 Spherocytosis	-	-	-	-	-	+	O+ve	-
31. M.S.	70	9.5	- Spherocytosis	-	-	-	Normoblastic hyperplasia	1.5	+	O+ve	11
32. N.S.	64	22.1	30 Spherocytosis	0.55	0.30	0.425	-	1.5	+	A+ve	11.5
33. S.S.	81	8	5 Spherocytosis	-	-	-	-	-	+	O+ve	-
34. D.W.	86	5.5	- Spherocytosis	0.55	0.40	0.425	-	-	0	-	-
35. G.W.	47	20	10 Spherocytosis Normoblasts	0.85	0.46	0.615	Normoblastic hyperplasia	2.0	+	A+ve	4
36. W.W.	40	29	33 Spherocytosis Normoblasts	0.85	0.48	0.595	Normoblastic hyperplasia	3.4	++	O+ve	15

a haemolytic crisis). In the whole series, the E.S.R. value ranged from 2 to 75 mm. in the first hour, the mean being 19.5 mm.

Spherocytosis was seen in films of the peripheral blood in all but four patients and normoblastaemia was frequently present; the morphological appearance of the red cells correlated well with the results of osmotic fragility tests (Table 16) in the eighteen subjects for whom these data were obtained; the exception is Case 60 who showed markedly increased osmotic fragility but in whom spherocytosis was not convincingly present in blood films. It was only in the four patients not showing spherocytosis in blood films (Cases 56, 59, 60 and 64) that osmotic fragility tests were held to contribute towards the diagnosis. The indication for osmotic fragility tests after twenty-four hours' auto-incubation of the patient's blood was discussed on page 36 and was used to advantage in Cases 59 and 64.

The bone marrow was examined in twelve patients and usually showed the expected normoblastic hyperplasia; however, in three subjects (Cases 52, 53 and 62) definite megaloblastic erythropoiesis was present. One of these (Case 52) had a serum vitamin B₁₂ level of 80 µg./ml. and has been described in the literature (Delamore, Richmond and Davies 1961). The implication of folic acid deficiency in the haematological findings in Cases 52 and 53 is discussed on page 181.

Acholuric/

Acholic jaundice was almost always present; it will, however, be seen from Table 16 that in three patients the serum bilirubin level was below 1 mg./100 ml. The serum bilirubin level was never high, being in the range 0.5-4.5 mg., mean 2.1 mg./100 ml.

In the twenty patients for whom a result is available, the direct Coombs' test was never positive. The data also suggest that there is no significant preponderance of a particular ABO blood group; there were thirteen patients in Group O, five in Group A and two in Group B.

Red cell survival was measured using chromium-51 in thirteen of the patients; the range for the $T_{1/2}^{Cr}$ was four to sixteen days, mean 10.4 days.

5. 4. Modes of presentation of disease

Since the clinical manifestations of congenital spherocytosis are protean, and since the severity of the haemolytic process may vary from being so mild that it can be easily overlooked to being so intense that it is life threatening, the many modes of presentation of the disease that have been encountered in this series of patients will be described briefly.

With chronic anaemia

Case 52 (J.C., 21 years) had a mild attack of jaundice at the age of twelve years; no information is available that can establish the nature of this illness but it was of short/

short duration and he was absent from school for less than two weeks. His health before this and subsequently had been excellent until the age of eighteen years when there was fairly sudden onset of weakness and lightheadedness. These symptoms did not subside during four days rest in bed and he was afterwards admitted to the local hospital; anaemia was discovered and he gradually improved during a two month period on iron therapy. During the next two years the boy was in moderately good health but tired easily, and then dizziness and fatigue gradually returned. There was some improvement while taking iron tablets but the same symptoms recurred two months later and were associated with considerable pallor and breathlessness; on this occasion a dragging sensation, worsened by deep respiration, was felt in the left hypochondrium for several days. Further iron therapy and several weeks' rest appeared to restore his fitness, but three months later, after a respiratory infection, considerable weakness and pallor led to his admission to hospital in Edinburgh and to the diagnosis.

A similar story, with symptoms of chronic anaemia and exacerbations of anaemia with or without a history of jaundice extending back for several years, also characterised the mode of presentation of Cases 58, 61 and 67.

With acute anaemia.

Case 75 (G.W., 48 years) would admit to no past ill-health other than occasional respiratory infections when he was first seen. He had suddenly developed acute breathlessness/

breathlessness on exertion and effort angina one week earlier; these symptoms had been particularly noticeable on stairs in his work as a Corporation meter reader. The patient had considerable anaemia, Hb. 47%, reticulocytosis 20%, marked spherocytosis, acholuric jaundice and easily palpable firm splenomegaly; the last suggested that an active haemolytic process had been present for some time. No family history of anaemia or jaundice was available, there was no evidence of spherocytosis in the patient's widower father or any of his five siblings. The patient's direct Coombs' test was negative and the diagnosis of congenital spherocytosis was established when definite spherocytosis and splenomegaly were found in his only son (Case 74).

A haemolytic crisis was also the mode of presentation in five other patients (Cases 53, 54, 55, 63 and 68). Case 53 showed megaloblastic erythropoiesis and is discussed below. Cases 54 and 55 were brother and sister; they had a severe haemolytic crisis within a few days of each other.

Case 54 (J.C., 7 years) was admitted to hospital on 25.6.60. His earlier health had been excellent and seven days prior to admission he had complained of a throbbing headache; the headache continued and became associated with increasing pallor, listlessness, anorexia and fever. The urine and stools became darker in colour. The spleen was palpable. The patient's initial haemoglobin level was 18%, the reticulocyte count was 2% and subsequent investigation showed/

showed marked spherocytosis, normoblastic hyperplasia in the bone marrow, acholuric jaundice, and negative Coombs' test. Case 55 (M.C., 4 years) was admitted to hospital on 2.7.60. She had been disinclined for food and on the day prior to admission she had become very pale and exhausted; her previous health had also been excellent. The clinical picture was similar to that found in her brother. The initial haemoglobin level was 37%, reticulocytes less than 1%. The fact that there was no evidence of bone marrow aplasia in either of these children may be due to the delay of nine days in sampling the marrow in the first, and five days in the second. Diagnosis was not difficult in this instance because their father was known to have had a splenectomy for haemolytic anaemia ten years earlier and there was a striking history of the disorder in other relatives (p.206).

There are many accounts of this interesting phenomenon of haemolytic crises occurring in different members of the same family more or less simultaneously. The first of these is believed to have come from Edinburgh (Murray-Lyon 1935) and attention was also drawn in this report to the occurrence of reticulocytopenia at the height of the crisis when anaemia was most severe. It is of interest that Owren (1948) based his observations on erythroid hypoplasia as the mechanism of production of crises on studies in six patients, four of whom were members of one family who became/

became ill within days of each other. The development of "familial crises" strongly supports the suggestion (Greig, Metz, Bradlow, Theron and Morris 1958, Wright and Gardner 1960) that an environmental factor is implicated in precipitating the event in some patients, and an infection seems the most likely. Although a history of infection was not available in Cases 54 and 55 it is notable that these were the only two in the series of patients whose E.S.R. exceeded 50 mm. in the first hour at the time of presentation.

With megaloblastic anaemia

Reference has already been made to Case 53 (F.C., 42 years) because of his presentation with acute anaemia. His past health had been good but there was known to be a high incidence of congenital spherocytosis in his family. One month before the patient was first seen he had developed an acute febrile illness with generalised myalgia and headache. Although the fever subsided in two to three days, the patient had remained weak and lethargic, and at the time of examination he showed considerable pallor, icterus and slight splenomegaly. The initial examination of peripheral blood gave the following results:- Hb. 45%, R.B.C. 2.0 m. per c.mm., reticulocytes 2.2%, W.B.C. 6,400 and normal numbers of platelets in the blood film. Acholuric jaundice was confirmed biochemically, the serum bilirubin level being 1.5 mg./100 ml.

The/

The patient was unable to accept hospital admission for a further month. By this time the haemoglobin level had fallen to 32% and a leucopenia of 800 white cells per c.mm. (with normal differential count) had appeared. The leucopenia was confirmed, being 1,000 white cells per c.mm. four days later, and at this time thrombocytopenia (platelets 15,000 per c.mm.) was also found to be present. Although the bone marrow showed normoblastic hyperplasia, megaloblasts were undoubtedly present. Prior to splenectomy, folic acid 5 mg. t.i.d. was given and within two days the white count was raised to 7,000 per c.mm. and the platelet count to 130,000; splenectomy was undertaken after three days and the usual rise in white cell and platelet counts followed. Repeat marrow examination two weeks later showed that megaloblasts were no longer present.

The relevant studies of vitamin B₁₂ and folic acid metabolism on this patient, in relation to the discovery of megaloblastic erythropoiesis, later revealed the following:- the serum vitamin B₁₂ level had been subnormal at 80 µg./ml. (normal range by L. Leichmannii method with added cyanide, Girdwood 1960, is 140-1,000 µg./ml.); vitamin B₁₂ absorption (Schilling method) was normal; folic acid absorption and excretion test (Girdwood 1960) was normal - the urinary output of folic acid after a 5 mg. parenteral injection of folic acid was 1.95 mg. and after a 5 mg. oral dose 2.3 mg., Excretion Index 117.9%; hydrochloric acid was/

was produced in the patient's gastric juice on maximal histamine stimulation; serum folic acid assays were not available at the time this patient was studied.

In Case 62 megaloblasts were also found in the bone marrow during the period of investigation; in this patient no cytopenia was evident, extensive studies of pathogenesis were not undertaken, the serum vitamin B₁₂ level was 715 $\mu\text{g.}$ /ml. and folic acid supplements were given empirically prior to splenectomy. Data are available, however, for a third patient (Case 52) who developed megaloblastic change in the bone marrow while under observation.

Case 52 had presented with chronic relapsing anaemia in the previous three years and has been mentioned above. When first seen, he had a haemoglobin level of 61%, reticulocytes 17.3%, white cell count 6,100 with normal differential count, platelets 240,000. Marrow aspiration showed normoblastic hyperplasia. Over the next ten days fall of the platelet count to 130,000 per c.mm. was associated with a fall in the reticulocyte count to 4%; at this time megaloblasts were definitely seen in a second marrow smear. Folic acid was given in a dose of 5 mg. t.i.d.; this reversed the thrombocytopenia and reticulocytopenia as is illustrated in Fig. 27. In this patient it was subsequently shown that the serum vitamin B₁₂ level had been normal at 633 $\mu\text{g.}$ /ml. but that the serum folic acid level had been below the normal range of 4.5 to 15 $\mu\text{g.}$ /ml. at 4.1 $\mu\text{g.}$ /ml./

Mr. J.C. 21 yrs. CASE 52

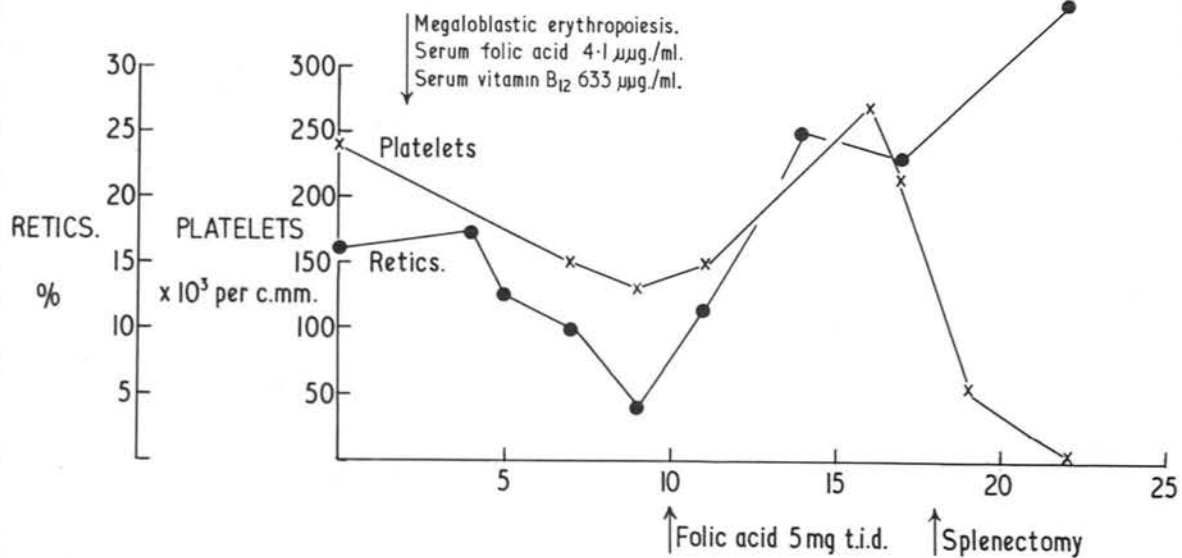


Fig. 27

The effect of folic acid supplements on platelet and reticulocyte counts in a patient with congenital spherocytosis who developed megaloblastic erythropoiesis accompanied by a subnormal serum folic acid level under observation.

ml.

The utilisation of haemopoietic factors in haemolytic disease was discussed on page 56 when the data regarding folic acid and vitamin B₁₂ metabolism for all patients were presented. The relative frequency of megaloblastic erythropoiesis is emphasised because it has now been described in three patients with autoimmune haemolytic anaemia and in a further three patients with congenital spherocytosis. Because of the good body stores of vitamin B₁₂ and its long biological half life, conditioned folic acid deficiency from increased haemopoiesis seems to be the more likely mechanism in most patients. This was the experience of Chanarin, Dacie and Mollin (1959). However, such may not always be the explanation since Case 52 did have a low level of serum vitamin B₁₂ without malabsorption of vitamin B₁₂, suggesting conditioned deficiency of this haematinic in his case. Moreover, conditioned deficiency of folic acid and vitamin B₁₂ may sometimes be the cause of an acute exacerbation of anaemia, particularly when leucopenia, thrombocytopenia and reticulocytopenia are present (as in Case 52); this cannot be the explanation of all crises in haemolytic disease, however, since Case 7, with idiopathic autoimmune haemolytic anaemia (p. 108), had a haemolytic crisis while she was receiving supplements of folic acid and vitamin B₁₂.

With jaundice

Case/

Case 51 (G.B., 19 years) had an attack of jaundice when five years old but had had good health before and after that episode until the illness which led to the diagnosis of congenital spherocytosis. Nine weeks prior to admission to hospital he had developed epigastric pain; this had persisted fairly constantly and seemed to be worst after a long interval without food and was relieved by the taking of food. During this period the patient had been noticed to be jaundiced, although the appetite was good; there had been an increase in the depth of colour in the urine and in the stools. On examination icterus was clearly evident but there was no pallor; the spleen was moderately enlarged. The haemoglobin level was 91%, reticulocytes 12.7% (indicating well compensated haemolysis) and there was marked spherocytosis. The serum bilirubin level was 3.4 mg./100 ml. and the urine contained urobilinogen but no bile. The direct Coombs' test was negative. Spherocytosis could not be shown in the boy's parents or in his three siblings; an uncle had, however, had several attacks of jaundice of unknown cause in the past but could not be examined because of service overseas.

Acholuric jaundice was also the outstanding feature in the history of two other patients (Cases 56 and 72 in the series).

With gall stones

Case 60 (E.H., 42 years) had almost certainly suffered from/

from chronic anaemia since early adult life. At the time of diagnosis she was pale and icteric, there was considerable splenomegaly, the haemoglobin level was 65%, reticulocytes 12% and acholuric jaundice was confirmed to be present on biochemical investigation. However, anaemia was not the clinical feature which led to diagnosis. Two weeks prior to admission to hospital the patient had complained of acute pain in the right hypochondrium, nausea, vomiting and anorexia. She had had similar, though less severe, attacks for several years. The attack subsided with exacerbations over three to four days and subsequent investigation showed the presence of multiple facettted radio-opaque gall stones in the gall bladder. While these gall stones may have developed on the basis of pigment gall stones, their "mixed" nature indicated chronic cholecystitis from recurrent infection.

The incidence of gall stones in this group of patients is lower than that reported in other series. Of the twenty-two who have proceeded to splenectomy, cholelithiasis was evident in only five (23%). The only feature which appeared to have any bearing on this complication was duration of disease, the ages of those showing gall stones being respectively: Case 60, 42 years; Case 62, 40 years; Case 65, 48 years; Case 67, 52 years; Case 76, 29 years. With cholangitis following cholecystectomy for gall stones.

Case 76 (W.W., 29 years) enjoyed good health until the age/

age of twenty-six years when he developed severe epigastric pain, lasting four days, associated with sweating, vomiting, shivering and possible jaundice. There were recurrences of mild epigastric pain lasting for a day or two in the next two years, and then he suffered a very similar attack to the first; this was sufficiently severe to warrant admission to hospital, because perforation of a peptic ulcer was suspected. The incident was considered to be due to acute ulcer pain rather than perforation, particularly as pylorospasm was later demonstrated during barium meal examination. It is of interest that splenic enlargement was reported by the radiologist at this time but its significance does not seem to have been appreciated. He remained well apart from occasional upper abdominal pains for four months, and then recurrence of another severe attack was associated with jaundice; the diagnosis was considered to be infective hepatitis until cholecystography showed the presence of multiple non-opaque gall stones. While awaiting cholecystectomy, another severe attack of pain supervened, again associated with jaundice. After allowing time for some improvement, cholecystectomy and exploration of the common bile duct was undertaken; multiple small pigment stones were found and the gall bladder appeared healthy. The correct diagnosis was still not entertained and about two months after operation the patient again became ill with pallor, shivering, sweating, vomiting/

vomiting, right hypochondrial pain and tenderness.

Cholangitis appeared to be present and the condition quickly settled on a course of "Crystamycin". It was only at this stage that the presence of anaemia, reticulocytosis, spherocytosis; considerable splenomegaly and acholuric jaundice (serum bilirubin 3.4 mg./100 ml.) suggested congenital spherocytosis and the diagnosis was made.

A very similar course was followed by Case 65. He had recurrent attacks of biliary obstruction with cholangitis for a year after cholecystectomy before a haemolytic process was suspected.

With splenomegaly and splenic infarction

Case 57 had been found to have splenomegaly during a routine school examination at the age of eleven years. She had intermittent mild anaemia during puberty and adolescence but this produced no more than mild disability and the diagnosis of congenital spherocytosis was not reached until the age of twenty-three years.

Case 71 (M.S., 36 years) had been easily fatigued following the birth of her third child one year previously. The main reason for her presentation was, however, sudden severe pain in the left upper quadrant of the abdomen, leading to admission to hospital as a surgical emergency. A mass was felt in this region, the nature of which was uncertain; it was, however, believed to be an enlarged left kidney; accordingly pyelography was undertaken but this showed/

showed normal renal outlines. The mass seemed more likely to be spleen in the X-rays and the appearance of a friction rub during a subsequent attack of pain strongly suggested that the patient's symptoms had been due to splenic infarction. At subsequent splenectomy, a spleen weighing 620 g. was removed and the presence of infarction and perisplenitis was confirmed. The diagnosis of congenital spherocytosis was reached on the basis of anaemia, Hb. 70%, spherocytosis, reticulocytosis (9.5%), acholuric jaundice and family history. The patient's father (deceased) was stated to have had splenic enlargement and recurrent jaundice (p.206) and her three children were found to be affected.

With pregnancy

Case 66 (C.M., 18 years) was considered to have been anaemic after her first pregnancy, nine months before she was seen in the present studies. The reason for her examination was moderate anaemia (Hb. 76%) at the sixth month of gestation in her second pregnancy. More detailed investigation revealed reticulocytosis (9.2%), spherocytosis, urobilinogenuria and palpable splenic enlargement. Case 71 also developed symptoms in relation to pregnancy (p.188) but no data are available to show the degree of anaemia at the time.

Although Case 66 did not have marked anaemia when the diagnosis was made, the possible aggravating role of pregnancy in haemolytic disease has been known for some time; Wintrobe/

Wintrobe (1961) lists pregnancy with "cold", "emotion" and "fatigue" as a cause of exacerbation, while McElin, Mussey and Watkins (1950) found that if worsening of a haemolytic disorder were to occur in pregnancy it was more likely after the third month of gestation than before. In the light of earlier discussion on conditioned deficiency of folic acid in some patients with haemolytic disease (p. 184), the occasional exacerbation of anaemia during pregnancy is not surprising in view of the evidence that pregnancy may also be a cause of folic acid depletion (Chanarin, Dacie and Mollin 1959, Girdwood and Delamore 1961).

With family study

Finally there were six patients in the series (Cases 59, 64, 69, 70, 73 and 74) who had no symptoms, who were not known to have any abnormality and who were discovered to have congenital spherocytosis in family surveys.

5. 5. The results of splenectomy.

Introduction. The first splenectomy.

The unique feature in congenital spherocytosis is that apart from rupture of the spleen there is no other condition in which splenectomy is followed by such uniformly good results. Dacie (1960) reviews the evidence for failure of splenectomy in the literature, and considers that in some instances the diagnosis was probably congenital non-spherocytic anaemia, and in other acquired haemolytic anaemia; in those cases where the diagnosis was acceptable, the/

the cause of relapse appeared to be splenunculi or splenic fragments left behind at operation (Stobie 1947, Loeb, Seaman and Moore 1952, Edwards 1955, McKenzie, Elliot, Eastcott, Hughes-Jones, Barkhan and Mollison 1962), except in an unusual case described by Freund (1932) in which the liver was intensely congested at autopsy and may have taken over haemolytic activity.

According to Lord Dawson of Penn (1931) the first splenectomy for congenital spherocytosis was probably undertaken by Spencer Wells in 1887. Spencer Wells did the first splenectomy in England in 1865; his second and third splenectomies were performed in 1868 and 1875 but all of these were unsuccessful and the patients died. His fourth splenectomy concerned a young women, twenty-seven years old. From the age of nine this patient had been troubled by frequent attacks of jaundice and when she was fourteen she was growing "so large in front" that her mother was disturbed. At the age of twenty-five a large abdominal tumour was discovered which was thought to be a fibroid. After two years' observation the swelling was noted to be increasing in size; the attacks of jaundice had become more severe and were now accompanied by abdominal pain. Accordingly, it was decided to remove the tumour. Spencer Wells found that the mass was a large freely movable spleen which weighed nearly 12 lbs. After operation the patient made/

made a complete recovery. The diagnosis was not reached, however, for another forty years when the patient still showed spherocytes in the peripheral blood and increased osmotic fragility of the erythrocytes. Her only son had earlier developed haemolytic anaemia in childhood and then obstructive jaundice from cholelithiasis; he had also made a complete recovery following splenectomy (and cholecystectomy).

Effect of splenectomy on the haemolytic process

Dacie (1943, 1960) draws attention to the fact that mild bilirubinaemia and reticulocytosis may persist in some patients after splenectomy, and considers that this may indicate continuance of the haemolytic process. In only two patients in the present studies has the reticulocyte count exceeded 2% after operation; in one, Case 71, the count has frequently been between 2 and 3% during the six years of follow-up, although the patient has never been anaemic; in the other, Case 65, high values were obtained, ranging from 6 to 12%, during a period of investigation seven years after splenectomy. The patient was then found to have moderate anaemia, Hb. 75%, and obstructive jaundice. Red cell survival measurement was undertaken and gave a $T_{1/2}Cr$ of 24 days. Subsequent laparotomy revealed a biliary stricture (cholecystectomy and exploration of the common bile duct having been earlier performed in this patient) and careful search of the abdomen failed to reveal any splenunculi.

In the only data that are known to be available regarding erythrocyte life span after splenectomy for congenital spherocytosis (Motulsky, Giblett, Coleman, Gabrio and Finch 1955), it was shown that while this may be returned to normal in most instances, it remains significantly impaired, although easily compensated, in others.

Clinical cure of anaemia has been achieved with apparent arrest of the haemolytic process in all twenty-two patients so far treated by splenectomy in the present series. The four patients who have not proceeded to surgery (Cases 58, 59, 64 and 74) all have well compensated haemolytic disease, are not disabled and are under regular review. The $T_{1/2}Cr$ has been measured in eight patients in the present series at intervals varying from sixty days to seven years, after removal of the spleen. (It will be indicated on page 200 that red cell survival measurements within a few days of splenectomy may show that excessive red cell destruction is still persisting.) The results obtained were as follows:- Case 53, 28 days; Case 60, 24 days; Case 61, 30 days; Case 63, 24.5 days; Case 65, 24 days; Case 72, 26 days; Case 75, 31 days; Case 76, 32 days. The mean value for the post splenectomy $T_{1/2}Cr$ in these eight subjects was 27.6 days. The data are illustrated in Fig. 28 and it has been concluded that the red cell survival time has been corrected in all of these patients, despite the persistence of spherocytosis in the peripheral blood.

The/

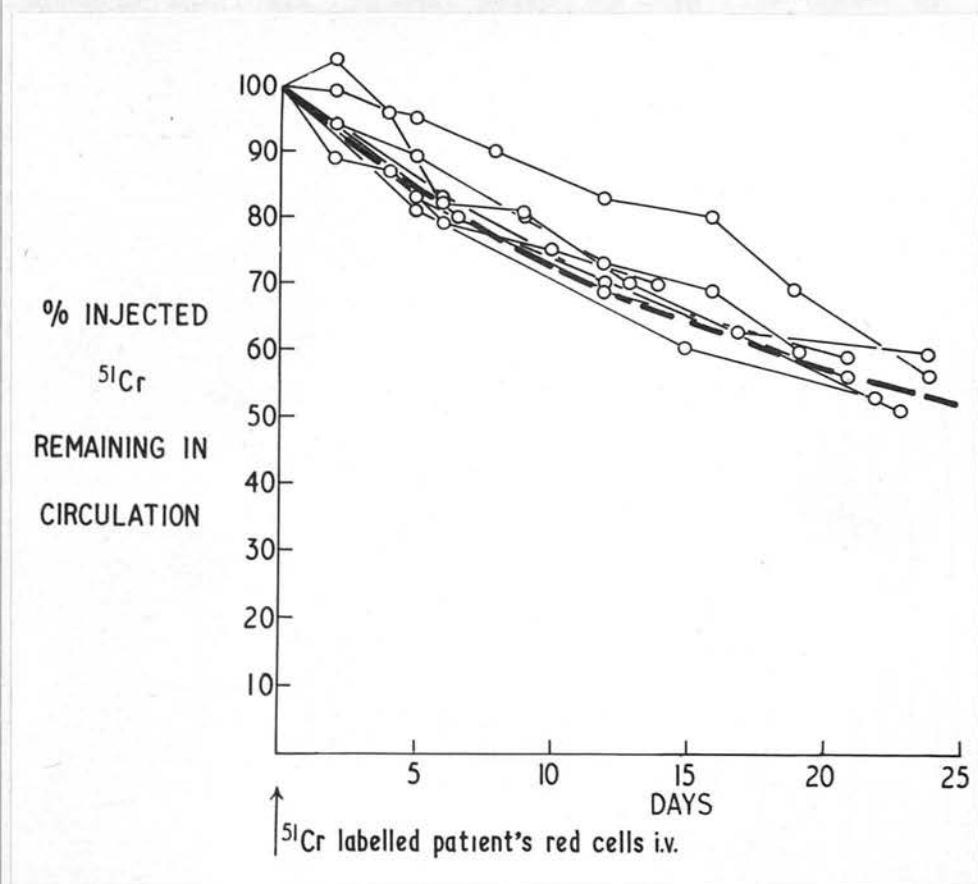


Fig. 28

Red cell survival after splenectomy in 8 patients suffering from congenital spherocytosis.

The remarkable effect of operation in congenital spherocytosis and the confirmation that this condition is indeed an intrinsic red cell defect is well illustrated by the cross transfusion experiments shown in Fig. 29. First, in Case 76 it is seen that the patient's erythrocytes have a shortened survival time in the circulation of a healthy recipient, as well as in his own; in fact, the cells appear to be destroyed more rapidly in the normal individual than in the patient, the $T_{1/2}^{Cr}$ being 8 days in the former and approximately 15 days in the latter. In Case 72 the patient's erythrocytes after splenectomy now have a normal survival time in himself (compared with a $T_{1/2}^{Cr}$ of 11.5 days before operation), but when injected into a healthy subject with an intact spleen, they are still removed from the circulation abnormally rapidly. Similar results have earlier been obtained by Dacie and Mollison (1943), Schrumph (1951) and Hughes-Jones and Szur (1957). Emerson (1954) went further and demonstrated that the patient's cells were incapable of normal survival in a normal subject except in the absence of a spleen.

Selection of patients for splenectomy and complications of surgery.

In view of the tendency to crises of anaemia and to the development of gall stones, and because of the good effect of splenectomy in congenital spherocytosis, it is generally recommended that surgery should be undertaken in all cases except/

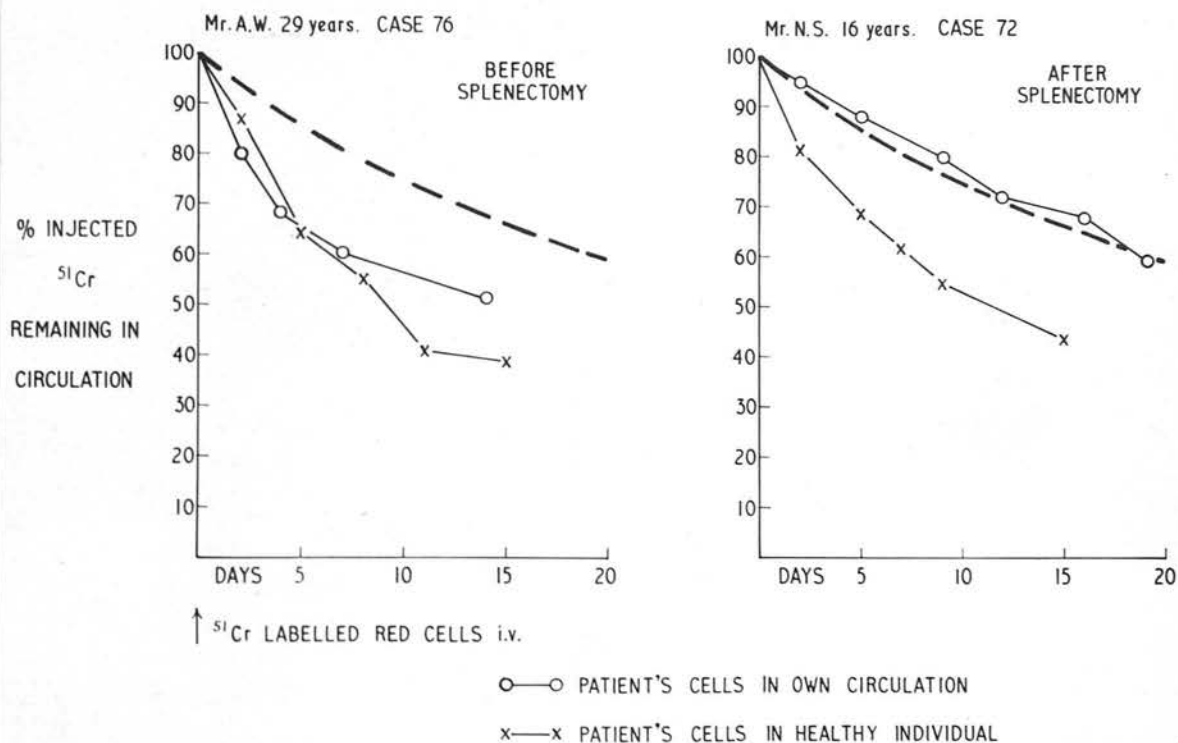


Fig. 29

Cross transfusion experiments in patients with congenital spherocytosis. The cells of Case 76 have a shortened survival in a normal individual and in his own circulation, illustrating that this condition is due to an intrinsic red cell defect. After splenectomy red cell survival is normal in Case 72 but his cells still have a shortened survival in a healthy recipient.

except in those few who are having no symptoms from the disorder and have well compensated haemolytic process. This has been the policy in the present series and as stated already, has meant that twenty-two of the twenty-six patients have had their spleens removed. One patient (Case 62) died two days post-operatively from unrecognised acute gastric dilatation and the sudden aspiration of copious vomitus into the lungs. The only morbidity from operation was seen in Case 75 who developed a deep venous thrombosis in one leg ten days after splenectomy, in association with a transient increase of the platelet count to 900,000 per c.mm.

It has been found necessary to recommend splenectomy in six children; in Cases 54, 55 and 68 because of a severe haemolytic crisis, in Cases 69, 70 and 73 because of failure to thrive and backwardness at school. These children have now been followed for periods varying from four months to eighteen months and are doing well.

There is, however, misgiving about the possible disposition to infection in children after splenectomy, and for this reason attempt is made to defer surgery for as long as possible. King and Schumaker (1952) were the first to draw attention to the problem; they reported five cases of congenital spherocytosis requiring splenectomy in the first six months of life; in all of them severe infections, usually meningitis, later developed and two died. Smith, Erlandson/

Erlandson, Schulman and Stern (1957) showed the risk was not restricted to splenectomy in infancy, describing infections after splenectomy in children and young adults. Huntley (1958) then indicated that the danger was not confined to splenectomy for any particular disease. Some authorities (Miller and Hagedorn 1951) deny an increased risk of infection, but this view is not shared by Lowdon, Walker and Walker (1962). The latter authors, reviewing the literature and analysing their own experience following splenectomy undertaken for various reasons in seventy-five children (four of whom developed serious infections), concluded that splenectomy may predispose to life-threatening infection in some cases, and such infection usually occurs within two years of operation. The risk, although present at all ages, is probably greatest if operation is performed in infancy. When infection occurs it is often due to the pneumococcus and it may take the form of a fulminating septicaemic illness with meningitis and the danger of adrenal haemorrhage. The account of Lowdon and his colleagues is the only one in British literature.

5. 6. Possible role of the spleen in congenital spherocytosis.

"Conditioning" of spherocytes

Although the place of splenectomy in the management of patients with congenital spherocytosis is unequivocal, the precise/

precise function of the spleen in the haemolytic process has not yet been established. Characteristically, the spleen is enlarged but there is no primary abnormality of the spleen, since it has been amply demonstrated, both in vivo (p. 194) and in vitro (Young, Platzner, Ervin and Izzo 1951), that a spleen from a patient with hereditary spherocytosis and one from a normal individual will both selectively remove normal red cells.

Numerous investigations (Dacie 1943, Young and his colleagues 1951, Weisman, Ham, Hinz and Harris 1955 and Emerson, Shen, Ham, Fleming and Castle 1956) have indicated that this sequestration of spherocytes by the spleen has a deleterious or "conditioning" effect on the cells. The changes are recognised by abnormally increased osmotic and mechanical fragility in blood taken from the spleen pulp or splenic veins; the "conditioned" cells may then either be destroyed in the spleen, as evidenced by the elevated bilirubin level in splenic vein blood (Gripwall 1938, Dacie 1960), or escape into the peripheral circulation where they are identified as the markedly fragile "tail" of cells in osmotic curve (p. 35). It has long been known (Meulengracht 1922, Dacie 1943) that these most spheroidal cells and most fragile cells are usually no longer present in the patient's circulation after splenectomy. This finding has been confirmed in six of the present series of patients and the data are illustrated in Fig. 30.

These/

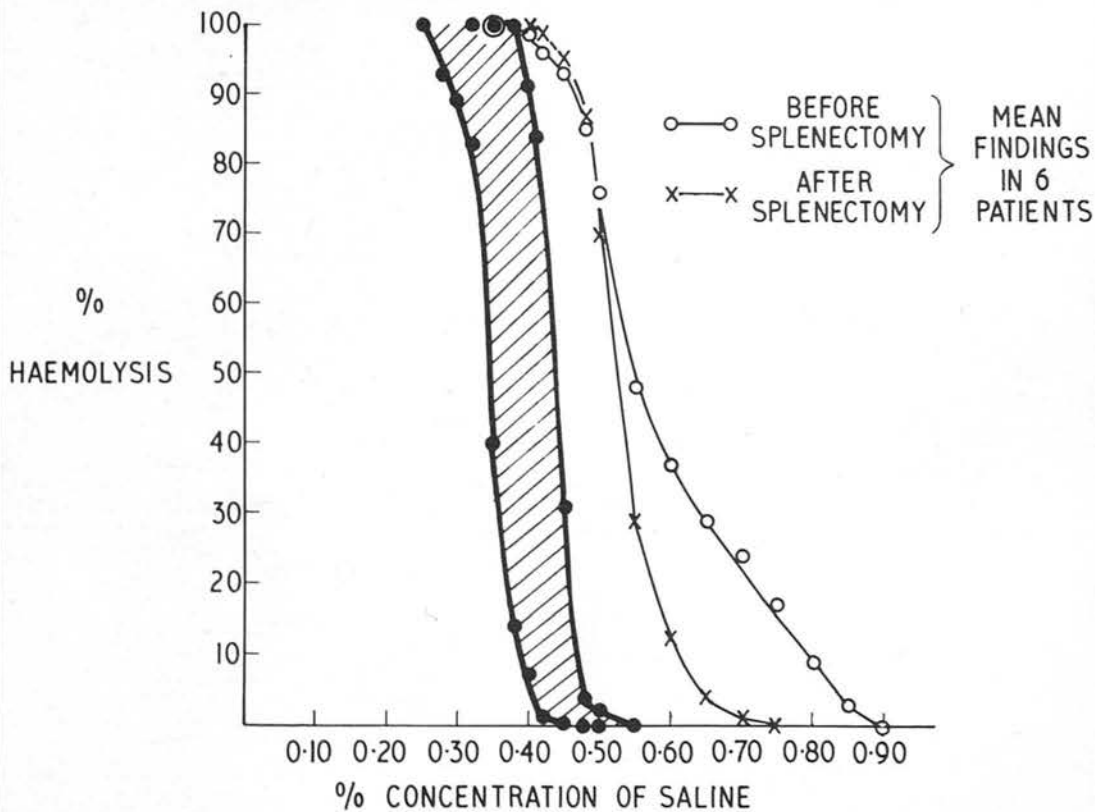


Fig. 30

Osmotic fragility of red cells in congenital spherocytosis. Note that the "tail" of the pre-operative curve due to the characteristic population of very fragile cells has been significantly altered by splenectomy.

These several observations have revived an old suggestion (Whipple 1941) that differential selection of erythrocytes by the spleen might occur on the basis of a geometrical peculiarity; the spheroidal cells may be easily trapped in the spleen because of their abnormal thickness and their inability to escape through the slit-like openings of the venous sinusoids; this is supported by the work of Bjorkman (1947) who found that animal spleens selectively retain particles ranging from 3-4 μ . in diameter. Crosby and Conrad (1960) reported observations, however, which make it unlikely that it is the shape of the red cell of congenital spherocytosis which is implicated. They removed blood by venesection from two healthy patients with congenital spherocytosis and compensated haemolytic disease until they developed iron deficiency and their red cells became hypochromic; in consequence the red cells became smaller and thinner, the resistance of the cells in tests of osmotic and mechanical fragility improved and autohaemolysis during incubation was greatly diminished. Modification of the red cell characteristics, relating to size, shape and iron content, was not, however, reflected in improved red cell survival.

Griggs, Weisman and Harris (1960) made an important contribution by studying the behaviour of an identifiable group of spherocytic red cells using in vivo labelling with radioactive iron (^{59}Fe). It is well established that iron administered/

administered in this way is incorporated only into newly formed red cells and not into older erythrocytes. These investigators found that the osmotic fragility of newly formed red cells was similar to the fragility of the main body of red cells in the peripheral blood; none of the new ^{59}Fe labelled erythrocytes were found in the tail of the osmotic curve, but then there was progressive increase in osmotic and mechanical fragility and it took at least ten days for "conditioning" to reach a maximum. It was impossible to say from the nature of the study whether the labelled erythrocytes were trapped in the spleen for the full period of "conditioning" or whether this change was achieved on multiple circuits. Then Griggs and his colleagues studied the survival of erythrocytes taken from the splenic pulp at the time of elective splenectomy in the patient's circulation. They found that the erythrocytes obtained from the spleen showing the most marked fragility had an extremely short survival and disappeared from the circulation in forty-eight hours. Motulsky, Casserl, Giblett, Broun and Finch (1958) performed a complementary experiment to the foregoing by transfusing ^{59}Fe labelled spherocytes from a patient, who had had a splenectomy, into his brother, who also had congenital spherocytosis but had not yet proceeded to operation. Approximately six days were required for these transfused cells to show the same marked increase in osmotic fragility as was present in the recipient's/

recipient's own cell population.

Studies supporting the conditioning role of the spleen.

Additional studies have been undertaken in the present series of patients. First, in two subjects (Cases 52 and 72) serial measurements of red cell survival have been made using chromium-51, before splenectomy, in the immediate post-operative period (within 48 - 72 hours), and at a later interval of 80 and 60 days respectively. The data are presented in Fig. 31.

The shortened survival time before operation is seen in each patient, as is the return to a normal survival time of the patient's cells by the time of the third measurement two to three months after splenectomy. The form of the survival curve in the immediate post-operative period indicates, however, that at this stage red cell survival is not yet corrected and that there still remains in the circulation a proportion of cells that are abnormally sensitive to destruction, and that these have to be cleared before the haemolytic process is completely arrested. Dacie (1960) has also shown in one patient that the rate of haemolysis remained unchanged for three days after splenectomy before becoming approximately normal.

Secondly, in eight of the patients body surface measurements of radioactivity during red cell survival estimates with chromium-51 were undertaken as in the patients with autoimmune and symptomatic non-immune haemolytic anaemia/

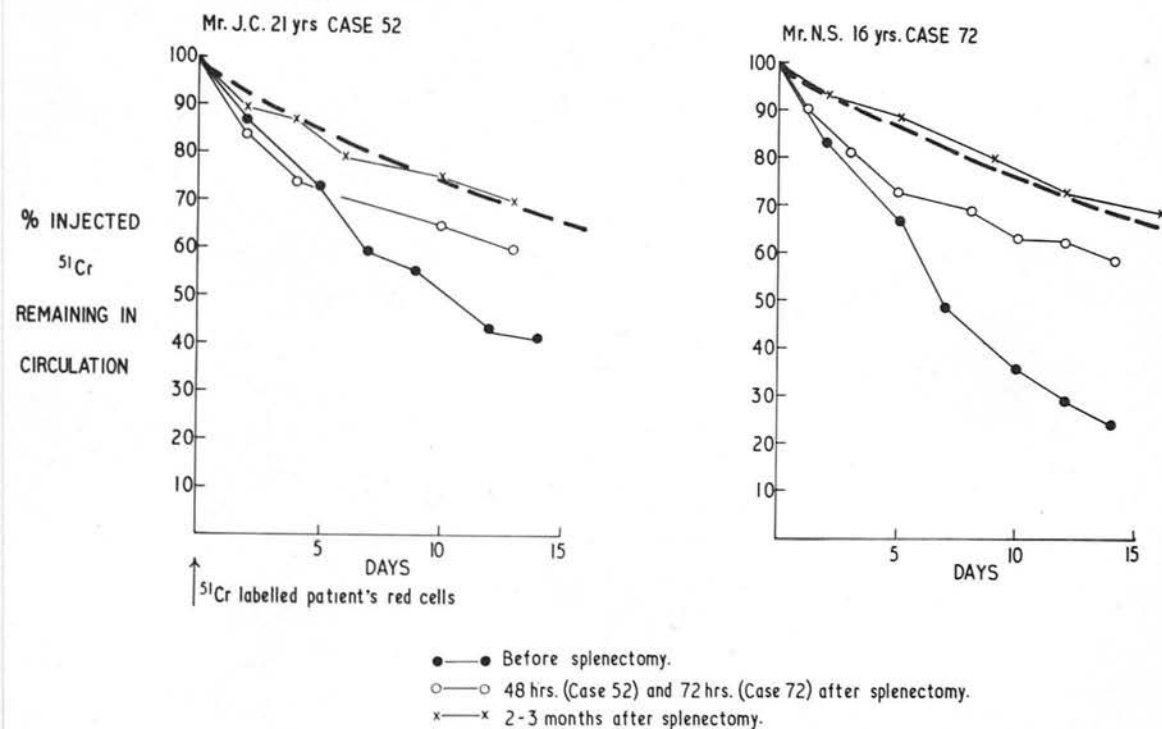


Fig. 31

Serial measurements of red cell survival in two patients with congenital spherocytosis. The haemolytic process is not immediately corrected by splenectomy, presumably due to persistence of a proportion of excessively fragile cells in the circulation for a few days after operation.

anaemia. The data are presented in Table 7 and are illustrated for seven of the patients in Fig. 32.

It is seen that the spleen sequestration index ranges from 40 to 265, mean 153, and the spleen/liver ratio at $T_{1/2}Cr$ from 1.7 to 3.3, mean 2.42. The Spleen Number which has been used as the most sensitive indicator of splenic sequestration ranges from 82 to 585, mean 371.

These values and visual inspection of Fig. 32 show that, while in most patients there is a significant accumulation of radioactive chromium in the spleen, this is not always so, and in any case it never reaches the very high levels of spleen radioactivity (p. 100, 152) encountered in patients with the other haemolytic diseases. For example, the Spleen Numbers of Cases 10 and 14, with idiopathic autoimmune haemolytic anaemia, were 734 and 753 respectively, and for Cases 27, 32 and 35, with symptomatic non-immune haemolytic anaemia, 920, 960 and 1056 respectively.

Study of the results of Jandl, Greenberg, Yonemoto and Castle (1956) and of Hughes-Jones and Szur (1957) also suggests that the uptake of radioactive chromium in congenital spherocytosis may not be as high as in cases of acquired haemolytic anaemia.

These observations are in good accord with experimental work recently reported by Belcher and Hughes-Jones (1960). These investigators made quantitative estimates of radioactivity in the spleen following splenectomy at an interval after/

TABLE 17

DATA REGARDING RED CELL SURVIVAL AND SPLENIC UPTAKE OF RADIOACTIVE
 LABELLED RED CELLS IN 8 PATIENTS WITH CONGENITAL SPHEROCYTOSIS
 USING CHROMIUM-51

Patient	51	52	58	64	67	72	75	76
G.B.	J.C.	M.F.	A.M.	M.R.	N.S.	G.W.	W.W.	
T _{1/2} Cr in days	9	10	14	16	13	11.5	4	15
Spleen Sequestration Index	40	100	185	135	225	195	265	80
Spleen/Liver ratio at T _{1/2} Cr	2.05	1.7	1.94	3.3	2.6	2.55	2.2	3.05
Spleen Number	82	170	359	445	585	497	583	244

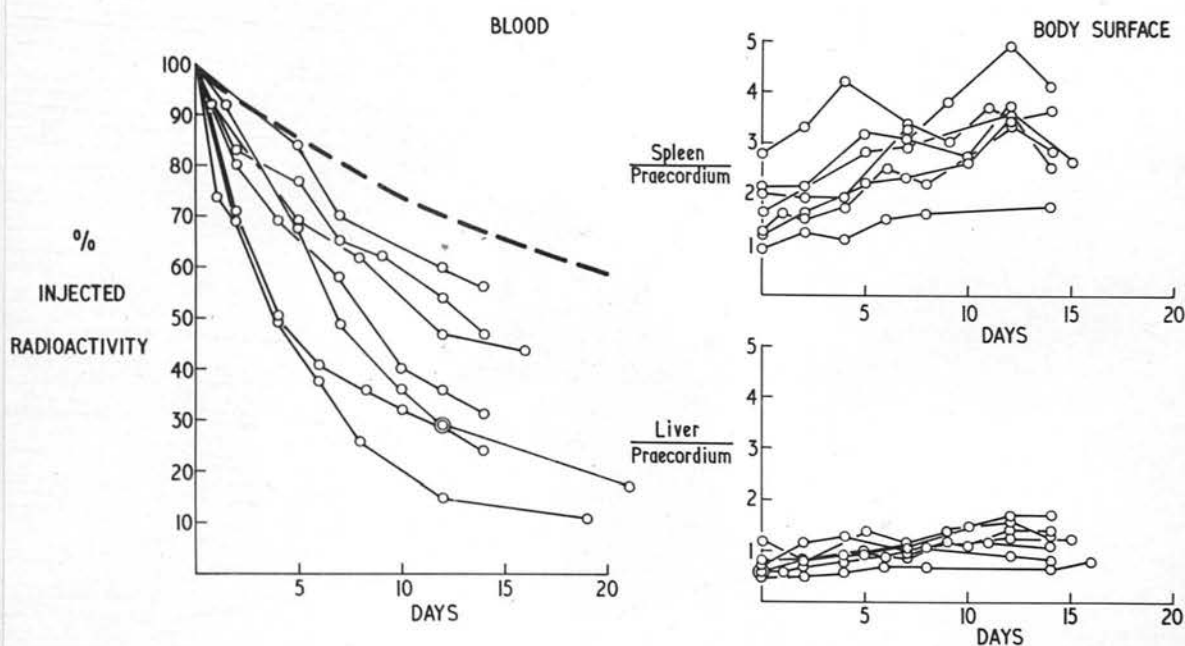


Fig. 32

Data regarding red cell survival and uptake of radioactivity from ^{51}Cr labelled red cells in 7 patients with congenital spherocytosis. The main feature is the relatively low uptake of radioactivity in the spleen in some patients compared with that seen in a proportion of the patients with acquired disease.

after ^{51}Cr labelled cells had been injected for the purpose of red cell survival measurement. They took account of the rate of random destruction and the potential life span of the red cells and made assumptions relating to the rate of elution of ^{51}Cr from red cells in the circulation and the rate of elution of ^{51}Cr from the spleen once it had been deposited there. In one patient with acquired haemolytic anaemia the injected ^{51}Cr appeared to be almost entirely deposited in the spleen and haemolysis ceased after splenectomy; in another with acquired haemolytic anaemia the result indicated that the spleen was only partially responsible for the destruction of labelled cells, and this was consistent with body surface radioactivity measurements which had earlier shown an uptake of radioactivity by both the liver and the spleen. In each of two patients with congenital spherocytosis studied, the spleen content of radioactivity was relatively lower than the first patient with acquired haemolytic anaemia, and it was therefore believed that the spleen was only partially responsible for the destruction of labelled cells in this disease.

When all these data are considered together, particularly the correction of osmotic fragility after splenectomy, the delay in correction of the haemolytic process after splenectomy and the relative splenic sequestration of ^{51}Cr labelled red cells in patients with congenital spherocytosis, compared with that seen in some patients with acquired haemolytic/

haemolytic disease, it is concluded that the main role of the spleen in congenital spherocytosis is to trap the spherocytes and critically increase their spheroidicity and fragility rather than to destroy them; while some red cell destruction takes place in the spleen, it seems likely that the "conditioned" erythrocytes are removed from the circulation in different parts of the body.

5. 7. Hypocholesterolaemia in congenital spherocytosis

An incidental finding

During the course of study of this series of patients incidental observations have been made of low levels of plasma cholesterol in some patients with untreated congenital spherocytosis. The first of these was made in Case 71 (M.S., 36 years); this patient had a plasma cholesterol of 133 mg. per 100 ml. (the normal range is 125-300 mg./100 ml.). The next was in Case 76 (W.W., 29 years); his plasma cholesterol was 51 mg. per 100 ml. (mean of two readings, 62 and 40 mg./100 ml.). After splenectomy the respective values of plasma cholesterol for these patients were 275 and 235 mg. per 100 ml.

The data regarding plasma cholesterol that are available are summarised in Table 18. Most but not all of the nine untreated subjects are shown to have a low value, while the eight splenectomised patients all have levels well within the normal range. The mean value before splenectomy is 156 mg./100 ml. and after splenectomy 242 mg./100 ml.

This/

TABLE 18

PLASMA CHOLESTEROL LEVELS IN PATIENTS WITH
CONGENITAL SPHEROCYTOSIS BEFORE AND AFTER SPLENECTOMY

Before Splenectomy		After Splenectomy	
Case No.	Plasma Cholesterol mg./100 ml.	Case No.	Plasma Cholesterol mg./100 ml.
51	118	53	192
52	119	57	285
58	170	60	237
59	264	63	207
64	173	67	258
71	133	71	275
72	100	75	250
74	276	76	234
76	51		
Mean	156		242

This is a subject for further study, and as far as is known the observation has not previously been reported in the literature. The suggestion is advanced that cholesterol is lost from the circulation with the bile which is excreted in excess, consequent on the haemolytic process, and this is corrected when excessive red cell destruction is arrested by splenectomy. In this regard it is notable that the four patients with the highest figures for plasma cholesterol before splenectomy (above 150 mg./100 ml.) are the four who have such mild well compensated haemolytic disease that operation has not so far been considered justifiable.

5. 8. The inheritance of congenital spherocytosis

Reference was made earlier (p. 166) to the inheritance of congenital spherocytosis and to the evidence indicating transmission of the abnormal gene as a Mendelian dominant with varying penetrance. The possibility of gene mutation as an explanation for "sporadic" examples of the condition was also stated but this had been considered unlikely by Race (1942) and Dacie (1960).

A number of observations relating to the different modes of inheritance have been made in the present series of patients.

In eighteen patients there was evidence of a dominant type of inheritance in living siblings and descendants. In twelve, disease was also found in a parent but in the other/

other six this was not so either because the parents were dead and the history was negative (four cases), or because the examination of the parent and his or her blood, including incubation osmotic fragility (p. 36), was negative (two cases).

In eight patients in the series no evidence of disease elsewhere in the family could be obtained. Three require special mention. Case 72 was an adopted child whose relations were not known. The parents of Case 60 were dead (but gave no history of illness), she was an only child and had had no descendants because of three miscarriages. The parents of Case 57 were examined with negative results; she was also an only child who had no descendants because of three miscarriages. Of the remainder, Case 68 was an only child and none had children. All available parents and siblings were examined for splenomegaly, anaemia, reticulocytosis and spherocytosis (by blood film scrutiny, standard osmotic fragility test and incubation fragility test) with negative results.

Three complete family trees serve to illustrate the main findings. Fig. 33 relates to Case 61 (G.I., 20 years). All four siblings were healthy as were the parents. All four grandparents were dead but had lived to ages exceeding sixty-five years and were never suspected of having any haematological disease. Fig. 34 relates to Case 63 (R.M., 12 years); there was no disease in her siblings or in her parents/

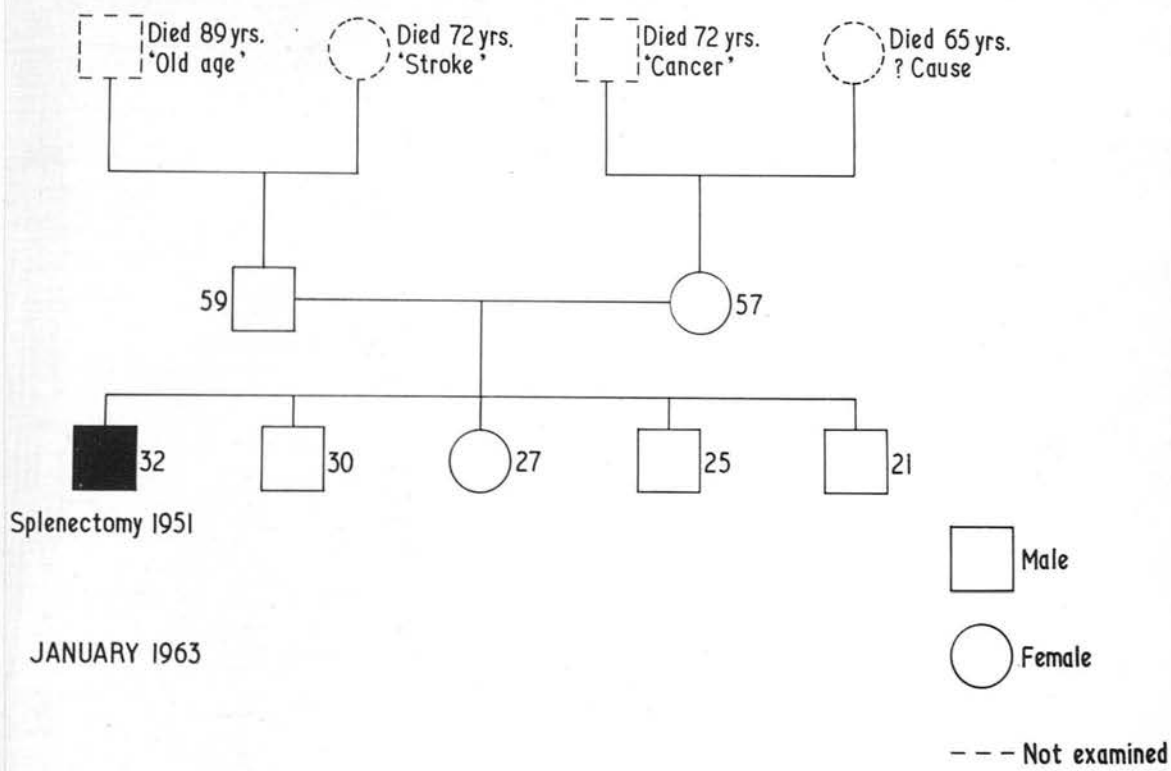
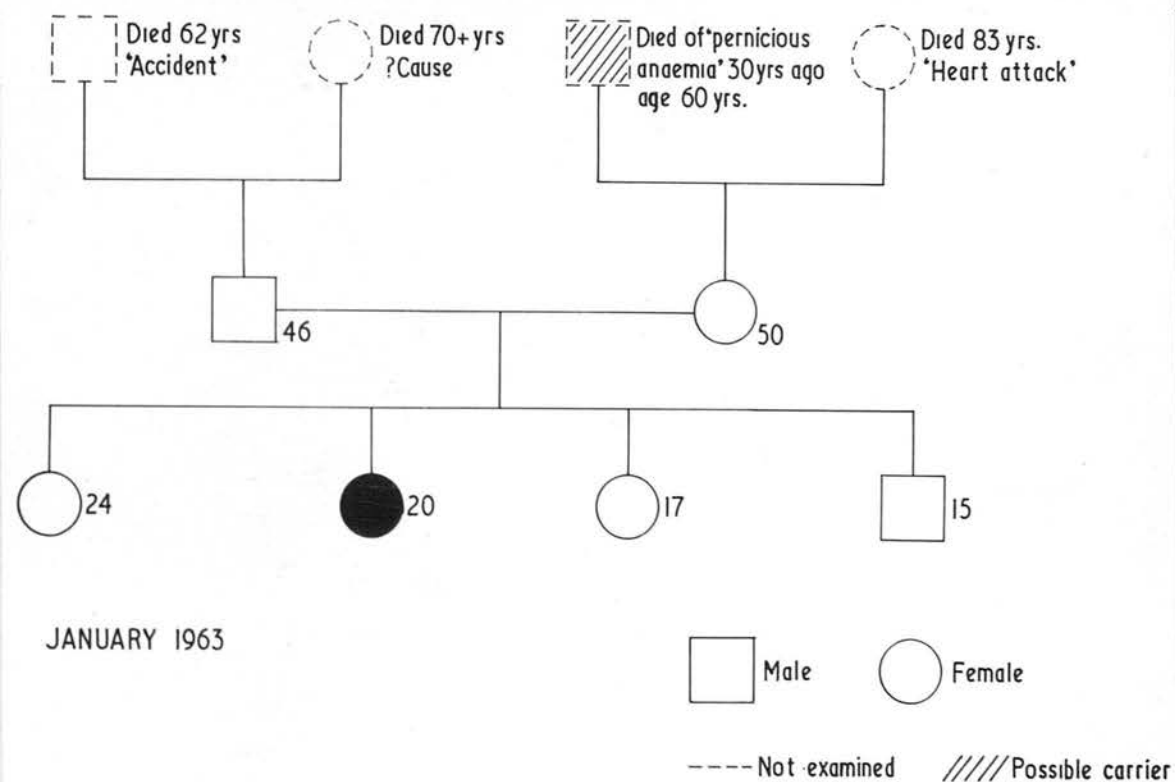


Fig. 33

The family tree of Case 61. No evidence of congenital spherocytosis was obtained in siblings or in two generations of antecedents.



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Fig. 34

The family tree of Case 63. No evidence of congenital spherocytosis was obtained in siblings or in two generations of antecedents. The maternal grandfather who died aged 60 years of "pernicious anaemia" may have been a carrier of the disease and the family may illustrate poor penetrance and expression of the abnormal gene.

parents; however, the maternal grandmother died at the age of sixty years of "pernicious anaemia". This death occurred thirty years ago and the diagnosis may well have been correct, but suspicion is raised that this individual was a "carrier" of congenital spherocytosis, illustrating the suggestion of Race (1942) and Young (1955) that expression of the abnormal gene in some families may be extremely feeble.

Fig. 35 illustrates the behaviour of a gene with very full expression and includes Cases 53, 54, 55, 56, 69, 70, 71 and 73. In the first generation there is little doubt that grandfather was affected as was one of his sisters, but both were dead before study could be undertaken. Grandfather had had recurrent attacks of jaundice throughout his life. He was examined in the Royal Infirmary, Edinburgh, in 1926 because of such an attack; the spleen was enlarged at the time and the diagnosis was considered to be biliary cirrhosis. The patient eventually died of a cerebrovascular accident at the age of seventy-three. Three of his children were affected, one presenting with chronic dyspepsia and jaundice, one with a splenic infarction and one with acute anaemia associated with megaloblastic erythropoiesis. Five of the third generation show the disorder; brother and sister of one family developed a haemolytic crisis together and the three children in the other family (one of whom is a Mongol), because of anaemia and/

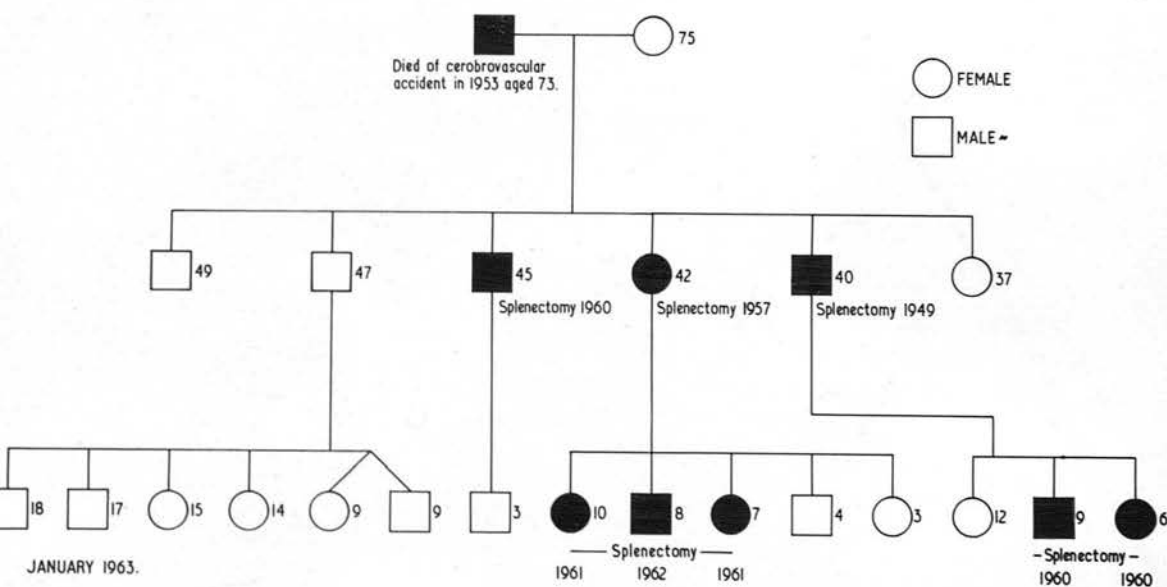


Fig. 35

A family with congenital spherocytosis in which the abnormal gene has behaved as a Mendelian dominant with full penetrance and expression. There is also evidence of advancing severity of the disease in succeeding generations.

and failure to thrive, have also required splenectomy. Not only does this family show the vagaries of the disease but it also illustrates advancement of the severity of the condition in succeeding generations. Although this phenomenon is well known in some inherited disorders, e.g. dystrophia myotonica, no reference has been found to its occurrence in congenital spherocytosis.

5. 9. Main conclusions from studies in congenital spherocytosis.

(i) Congenital spherocytosis has been studied in an unselected group of twenty-six patients. As in other series neither sex predominated, there being fourteen males and twelve females, and there was a wide range of age at the time of presentation, from eighteen months to sixty-two years.

(ii) Many modes of presentation were encountered; chronic relapsing anaemia, acute crisis of anaemia (in one instance occurring simultaneously in two members of a family), megaloblastic anaemia, splenomegaly, splenic infarction, jaundice, gall stones, cholangitis following cholecystectomy and anaemia in pregnancy.

(iii) There was a varying degree of anaemia, spherocytosis, reticulocytosis and acholuric jaundice at the time of diagnosis, and all but two patients had palpable splenic enlargement. Of the miscellaneous features, two were mentally backward (one was a mongol), one had a hare lip, two/

two had childless marriages, each having three miscarriages, and two had a chronic dermatosis affecting the legs above the malleoli.

(iv) Three subjects showed megaloblastic erythropoiesis. In two of these it was possible to reverse the thrombocytopenia and reticulocytopenia which accompanied the marrow changes by the giving of folic acid. Data are given suggesting that megaloblastic erythropoiesis may result from folic acid deficiency or vitamin B₁₂ deficiency or both.

(v) All but four patients (who had little or no anaemia and well compensated haemolysis) proceeded to splenectomy. One patient died in the immediate post-operative period; in the others clinical cure has been achieved. Six of the patients were children and splenectomy was advised because of a severe haemolytic crisis or because of chronic anaemia and failure to thrive. In follow-up periods ranging from four to eighteen months, there has been so far no incidence of serious infection.

(vi) In view of the lack of information regarding the red cell survival time after splenectomy for congenital spherocytosis, this measurement has been undertaken in eight patients. In all the T_{1/2}Cr was restored to normal, in the range 24 to 32 days.

(vii) Evidence is advanced to suggest that the role of the spleen in congenital spherocytosis is to "condition" the cells and to critically increase their spheroidicity and fragility/

fragility, rather than to destroy them. This is based on the improvement in osmotic fragility that succeeds splenectomy, the delay in arrest of the haemolytic process after splenectomy and on the measurements of splenic sequestration of ^{51}Cr labelled red cells. The last indicate that the uptake of radioactivity in congenital spherocytosis is never as high as is encountered in some patients with acquired haemolytic disease, and is in accord with absolute estimates of splenic sequestration made by others at the time of splenectomy.

(viii) Hypocholesterolaemia not previously reported, has been an incidental finding in untreated patients with congenital spherocytosis. The plasma cholesterol level was 51 mg./100 ml. in one patient and less than 140 mg./100 ml. in four others. The four patients with higher values than this had such mild haemolytic disease that splenectomy has not so far been found to be indicated. In measurements made after splenectomy, the plasma cholesterol level was normal and the suggestion has been advanced that this finding may be due to loss of cholesterol with the excessive amounts of bile that are excreted in haemolytic disease, correction being achieved by arrest of the haemolytic process.

(ix) Finally, studies of the mode of inheritance of the disease confirm the findings of others. In one family the gene behaved in a classical Mendelian dominant manner and showed increase in the severity of expression in succeeding/

succeeding generations. In eight patients no evidence of the disorder was found elsewhere in the family, but the influence of miscarriages and possible "carrier" states on this finding is discussed.

S U M M A R Y

1. The thesis has considered the investigation of haemolytic disease and has discussed observations on seventy-six patients regarding the pathogenesis, natural history and management of excessive red cell destruction in the three conditions that are most commonly encountered in Great Britain, namely autoimmune haemolytic anaemia, symptomatic non-immune haemolytic anaemia and congenital spherocytosis.

2. The clinical features and the diagnostic value of routine examination of the peripheral blood, bone marrow and urine for signs of anaemia, reticulocytosis and acholuric jaundice, the classical indicators of haemolysis, are described in each group. Where applicable, study has been made of the role of serological investigation and of tests of red cell fragility.

Since measurements of red cell survival using chromium-51 as an erythrocyte label have formed a major interest, they have been considered in detail. The technique has been examined for reproducibility, studied in normal subjects and various chronic and haematological diseases and compared simultaneously with the Ashby method. It was decided to use the $T_{1/2}^{Cr}$ (the time in days when 50 per cent of the injected ^{51}Cr has been cleared from the circulation) as the basis for comparison between data.

The/

The procedure for estimating the role of the spleen in a haemolytic process by measuring its uptake of radioactivity, due to sequestration of ^{51}Cr labelled red cells, has been widely applied. The spleen sequestration index (the percentage increment in radioactivity over the surface of the spleen between the start of the test and $T_{1/2}\text{Cr}$) and the spleen/liver ratio at $T_{1/2}\text{Cr}$ have been used, but neither adequately describes the implication of the spleen; the former ignores any liver uptake of radioactivity and the latter does not take account of high blood flow through the spleen. Accordingly a new measurement, the Spleen Number, a multiple of the spleen sequestration index and the spleen/liver ratio at $T_{1/2}\text{Cr}$ has been proposed.

3. In the group of patients with autoimmune haemolytic anaemia, apart from two subjects with the cold haemagglutinin syndrome, the nature of the red cell antibody has not clearly influenced the course of disease or its response to treatment. Blood transfusion has been used freely without incident; one patient required more than 40 litres in six weeks for survival. All patients received steroid therapy at some stage of their management; the dilemma that arises when response is poor or when the steroid preparation cannot be withdrawn to an acceptable maintenance level has been well illustrated; approximately one third of the patients had major complications from steroid therapy. Splenectomy was recommended in nine of the twenty-three patients; only one/

one of these had a good result from operation while three others had a partial remission. From the data that are available a high Spleen Number (exceeding 300) appears to offer prospect of remission from splenectomy; it is also of interest that high Spleen Numbers were seen in those who had the best remissions from steroid therapy.

The course of autoimmune haemolytic anaemia followed three paths; self limiting disease, chronic relapsing disease and fulminating, potentially fatal, disease. Three unique patients were considered in detail. The first died in infancy of a peculiar haemophagocytic reticulosis that had earlier killed two of his three siblings. The second was observed for several years; she was a good example of the chronic relapsing variety of illness and required steroid therapy only during exacerbations of anaemia; the haemolytic process was constantly active and during this time she had two normal pregnancies, in the second of which she was given large doses of prednisone in the first trimester without ill effects; she had two crises of anaemia, one while receiving folic acid supplements and one which was believed to be due to folic acid deficiency. The third patient developed a violent haemolytic process while being treated with prednisone for idiopathic thrombocytopenia, previously cured by splenectomy. Some influence on the condition was achieved by irradiation of the thymus; haemolysis was eventually arrested and the thrombocytopenia corrected by the/

the combined use of the new antineoplastic agents, Actinomycin-C and BW 57-322 (Imuran).

4. Studies on symptomatic non-immune haemolytic anaemia have been confined to patients with leukaemia, reticulosis and myeloid metaplasia. The usual signs of overt haemolysis, reticulocytosis and acholuric jaundice, were found in only one patient, despite markedly reduced red cell survival in many in the group. Neither splenomegaly nor its degree correlated with severity of red cell destruction or with the splenic uptake of ^{51}Cr labelled red cells; all grades of splenic sequestration were found, the Spleen Numbers ranging from 26 to 1056. Irradiation of the spleen caused a valuable reduction in haemolysis in some patients, but this effect was not related to the Spleen Number; the role of this form of treatment has been discussed.

Two patients in this group were subjected to autopsy shortly after red cell survival measurement with chromium-51 and it was possible to estimate the content of radioactivity in most body tissues. There was good agreement between the spleen/liver ratio at autopsy and that obtained earlier in vivo at $\text{T}_{1/2}\text{Cr}$. The data from one of the patients suggest that the marrow may be a much more important site of erythroclasis than is generally realised.

5. Congenital spherocytosis was encountered over a wide range of age from the first months of life to sixty-two years./

years. There were all grades of severity and many different modes of presentation; there was a small incidence of mental defect, developmental anomaly, miscarriages and chronic dermatosis of the legs and this has been described. Only five patients (23%) had gall stones. One simple point emerged that might be useful in the early steps towards diagnosis; the E.S.R. tends to be low in this condition and very high in autoimmune haemolytic anaemia, the only other haemolytic disorder which is commonly accompanied by spherocytosis.

Splenectomy has been undertaken in all but four patients who have mild well compensated disease. Removal of the spleen appears to have arrested haemolysis in all but one patient who died in the post-operative period; red cell survival has returned to normal in the eight patients in whom this has been measured. Observations have been made which support the view that the role of the spleen in congenital spherocytosis is to "condition" the red cells and critically increase their spheroidicity and fragility rather than to destroy them; these show improvement in the osmotic fragility of red cells after splenectomy, some delay in the correction of red cell survival after operation and lower Spleen Numbers than have been encountered in some of the patients with acquired disease.

Hypocholesterolaemia in untreated patients with congenital spherocytosis has been an incidental finding. The values/

values for plasma cholesterol after the haemolytic process has been arrested by splenectomy are normal.

6. Megaloblastic erythropoiesis has been observed in seven patients, four with autoimmune haemolytic anaemia (one in early pregnancy and one with a previous history of gastroenterostomy) and three with congenital spherocytosis.

While the available data suggest that conditioned deficiency of folic acid is most usually implicated and that the giving of folic acid supplements will correct leucopenia, reticulocytopenia and thrombocytopenia, if these be present, three of the patients (including the patient with previous gastroenterostomy) had low levels of vitamin B₁₂ in the serum.

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